THE INFLUENCE OF TRISTEZA ON CERTAIN BIOCHEMICAL CONSTITUENTS OF 'KEY' LIME (CITRUS AURANTIFOLIA)

By

KUNWAR BRIJENDRA PRATAP SINGH

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA
August, 1963

ACKNOWLEDGEMENTS

The author wishes to express his deep gratitude to Dr. R. H. Biggs, Assistant Biochemist, Co-chairman of the Supervisory Committee, under whose direction the work was carried out, for his valuable help and constructive advice during the course of the research and preparation of this manuscript.

Deep appreciation is also extended to Dr. A. H. Krezdorn, Head of the Department of Fruit Crops, Chairman of the Supervisory Committee, whose valuable advice and financial help were a constant source of inspiration.

With deep gratitude he acknowledges the advice and criticism of Dr. G. R. Noggle, Head of Botany Department; Dr. J. M. Soule, Jr., Associate Professor of Fruit Crops; Dr. Y. Sagawa, Assistant Professor of Botany; Dr. W. C. Price, Virologist; and staff of the Plant Virus Laboratory.

Thanks are also due to Dr. S. H. West, Plant Physiologist, Department of Agronomy for the use of the physical facilities and his continuing interest in the problem.

The writer would also like to convey his sincere thanks to Miss Mary C. Langan, Laboratory Assistant, Department of Fruit Crops; Mr. Simón Malo; and Mrs. Celia Lescano, for their technical help in the experiments reported in this dissertation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure

INTRODUCTION

Tristeza is a major disease of citrus. From the work of Oberholzer (97) in 1947, who discovered that the "incompatibility reactions" between the rootstock of sour orange and several scion varieties of citrus in South Africa was the result of a disease, it became evident that the causal agent must have been present there since about 1896. Although records are not complete enough to substantiate it, the disease probably reached South Africa from India not long after the colonization by the Dutch in 1562. It was not until 1924-25 that Webber (132) suggested from his observations that "tree failure" was caused by a disease and not incompatibility. Then, in 1933, Carrera (22) showed that a disease was prevalent in South America, which caused incompatibility between rootstocks of sour orange and several scion varieties of citrus. After this disease was first noticed in South America and in subsequent years killed millions of trees, it spread to California, Texas, Louisiana, and Florida (61). This disease was referred to as "tristeza of citrus" by Moreira in 1942 (92).

By 1944, the intensive programs were initiated to disclose the nature of this disease and to eradicate it (96, 120, 124). Two years later, Fawcett and Wallace (44) reported that the causal agent of "quick decline" or tristeza could be transmitted by grafting. In the same year, Meneghini (87) reported transmission of tristeza in Brazil by Toxoptera citricidus (Kirk.. In 1951, Dickson et al. (39) found that Aphis gossypii

Glover is the principal vector in California. Bennett and Costa (14) in 1949 confirmed Meneghini's results with aphids and also showed again that tristeza is transmissible by graftage. These reports indicated strongly that tristeza may be viral in nature.

Hughes and Lister (69) described veinal flecking and pitting of twigs in seedlings of lime and suggested in 1949 the use of West Indian lime as a biological indicator of tristeza. Then, in 1952, Grant (58) demonstrated the presence of tristeza or "quick decline" virus disease in Florida citrus, by grafting the infected material, Citrus aurantifolia Swing. Cv. 'Key' lime. With these grafting experiments and later ones, a great deal has been learned about the nature of the disease known as "tristeza of citrus," the reaction of certain varieties, species, and hybrids to this disease; and its symptomatology (52).

Though the experiments regarding transmission, symptomatology, and anatomical effects (117) of this disease suggested that it is viral in nature, it can not be confirmed until one establishes the form and function of this causal organism in vitro, as in the case of tobacco mosaic virus (TMV) and others which have been isolated and studied in vitro. In view of the fact that the causal organism of tristeza has not been isolated and studied in vitro, it would seem that a study of the physiology of the host under invasion by tristeza would be useful in helping to substantiate the viral nature of tristeza.

To accomplish the above objective, this study was made to determine the influence of tristeza on certain phases of the physiology of 'Key' lime seedlings. As no work has been done on the physiology of citrus

during the period of tristeza infection, the experiments were based on the information gleaned from a great deal of work that has been done on the relation between viruses and hosts of a number of other plant species, especially the investigations concerned with TMV, which have shown that it influences nitrogen, carbohydrates, and phosphorus metabolism of the host plants (11, 17, 43, 71, 139).

Therefore, this study included determinations of the influence of tristeza on soluble nucleotides, and certain other phases of nitrogen, carbohydrates, and phosphorus metabolism of 'Key' lime. Also, attempts were made to modify the influence of tristeza on 'Key' lime by gibberellic acid, a chemical which reverses dwarfism; and 2-thiouracil, an analogue of uracil which blocks the synthesis of viral-ribonucleic acid.

REVIEW OF LITERATURE

Symptoms of Tristeza in Citrus

Grant and Costa (60), Knorr (75), Cohen and Knorr (27), and Webber (132, 133) studied the symptoms of tristeza associated with different species, varieties, and hybrids of citrus in Florida. Busby (20) reported dulling or bronzing of the older leaves, suppression of growth, and patterns of minor element deficiency in foliage as an early syndrome of tristeza-infected plants. It was at a later infection stage that the leaves showed vein clearing, a condition characterized by single or numerous translucent elongated fleckings along the lateral veins. In some cases defoliation was also observed. In advanced stages the plants died. He also studied the different rootstock-scion combinations of citrus species, varieties, and hybrids and made a detailed list of tristeza-tolerant and susceptible rootstocks and scions of citrus.

'Key' Lime and Strains of Tristeza

'Key' lime is used as a biological indicator plant to index tristeza in citrus orchards (96). Grant and Higgins (62) found mixtures of strains of tristeza and has isolated individual strains. Grant (59), observing the syndrome of 'Key' lime infected with tristeza from selected sources, reported a variation in the severity of symptoms as a result of different strains of tristeza. Grant (59) also indicated

that 'Key' lime exhibited a range of symptoms when they were infected with mild and severe strains. Tristeza-infected plants showed stunting of growth of the main shoot and laterals, vein and veinlet clearing in the leaves, and pitting of the stems under bark.

Further, Grant (59) indicated that a severe strain of tristeza (T_3) caused vein clearing of young leaves which emerged soon after inoculation, and this symptom was followed by yellowing and cupping of the leaves. Grant also noted that 'Key' lime plants inoculated with infected leaf pieces showed scattered vein clearing in young leaves.

Effect of Other Viruses on the Metabolism of Host

As previously indicated, no work has been done to elucidate the abnormal physiology of the host as the result of tristeza infection; thus, it became essential to review the literature regarding the influence of other plant viruses, especially TMV and leaf-roll virus, on certain aspects of metabolism of the host. In 1931, Whitehead (135) mentioned that little attention had been directed to the metabolism of virus-infected plants. Wynd (139) reviewed the literature concerning metabolic phenomena associated with viral infection of plants. Since that time other interesting phases of altered metabolism as the result of infection by other plant viruses have been described by other workers (42, 65, 84, 99).

Nucleic acids and nucleic acid derivatives

Nucleic acid is an essential part of virus particles (55). Ribonucleic acid (RNA) is responsible for the biological activity in plant

viruses (28, 48). Crick (36) states with respect to the importance of nucleic acid: "These polymers appear to carry the pattern of living matter from one generation to the next. Their basic chain consists of sugar joined by phosphates, attached to the sugars in turn are bases."

It is clear from the work of Fraenkel-Conrat (46, 47) and Basler and Commoner (8) that the activity of the virus is associated with the polymer portion of nucleic acid and not with other small fractions of substances with high molecular weight. Fraenkel-Conrat (46) isolated native protein and ribonucleic acid by treatment with buffer at pH 10.5 and sodium dodecyl sulphate. At pH 6.0 mixtures of these almost non-infective components (10:1) were shown to reconstitute to an active virus. Basler and Commoner (8) suggested that excess nucleic acid which accumulated before TMV appeared represented the precursor of the polymer nucleic acid of TMV.

Ribonucleic acid, a regulator of biological reactions: Six criteria were formulated by Jacobs (70) for determining whether or not a substance normally controls a given biological process. Certain aspects of RNA in relation to biological reactions seem to indicate that it is a regulator, e.g., parallel variation, excision, substitution, isolation, generality, and specificity.

1. Parallel variation: Commoner and Basler (29) and Basler and Commoner (8) showed that among nucleic acids only RNA is present in plant viruses. RNA amounts differed from one strain of TMV to another. They mentioned that infection produced an increase in the nucleic acid content

of tobacco leaves, prior to the appearance of free TMV, so that it was higher than that of infected tissue. The increase was slightly in excess of the amount found in the TMV subsequently formed. During the synthesis of TMV, the excess nucleic acids progressively disappeared, until there was less nucleic acid in the infected tissue than in the non-infected tissue. Radioactive experiments indicated that nucleotides of a nucleic acid chain of viral particles determine the arrangement of amino acids in the polypeptide chain of protein of a virus particle (34).

Although these experiments supplied only qualitative data, it showed that the amount of infectious RNA varies in the intact organism in parallel fashion with the presence of chemical RNA which is also correlated to the growth and development of the host.

2. Excision: Loring (81) showed the quantitative effect of a powerful depolymerizing agent such as ribonuclease on ribonucleic acid. Inactivation of RNA either by ribonuclease (4) or thermal treatment inhibited the activity and multiplication of virus fragments. Loring (81) and Grant et al. (63) have obtained virus-free stocks by inactivating either by ribonuclease or thermal treatment, respectively.

Gierer and Mundry (54) proposed a tentative hypothesis for the chemical nature of the TMV on the basis of mutation caused by nitrous acid. The acid caused a rearrangement in the base-pairing in the RNA polymer so that the coding was changed. This in turn caused a change in the sequence of amino acids of the protective protein coat. The over-all result was the formation of a different strain of virus.

3. Substitution: In the case of plant viruses, active RNA is not like a phytohormone such as indoleacetic acid. It has no particular site of synthesis. RNA is present throughout the cytoplasm (5). At present very little substitution work has been done. Miller and Stanley (90) prepared the derivatives of TMV and tested them on Nicotiana glutinosa. These derivatives formed normal viruses.

4. Isolation: Fraenkel-Conrat (46) described a method of preparing infectious RNA. The material had an activity at 10 /uml. similar to that of TMV at 0.02 to 0.05 /uml. This lower degree of infectivity was probably the result of the fact that the RNA devoid of the protein is easily inactivated. This and similar experiments (47) have fostered the suggestion that the protein forms a protective coating over the RNA polymer.

A suggestion closely allied with this is the way in which the infection is initiated. In intact viruses, the nucleic acid moiety of the virus is freed from the protein moiety soon after entry into a cell of the host. Apparently, the exposing of biologically active core-RNA must be accomplished before virus multiplication begins. Ribonucleic acid without protein can initiate infection, but protein without RNA can not initiate the infection. Thus, a protein would seem to simply afford protection. However, it has not been proven that the role of protein is entirely a passive one (126).

5. Generality: De Fremery and Knight (38) and Knight (74) studied three strains of tomato bushy-stunt virus and TMV and found the content of nucleic acid to be about 16.5 and 5.0 per cent respectively. They

also suggested that RNA was responsible for viral infectivity. Cooper and Loring (35) and Markham and Smith (85) worked with eight strains of TMV and obtained similar results.

6. Specificity: It was not known clearly before 1956 that particles of nucleoprotein are responsible for viral infection. Since then it has been shown that the multiplication stimulus is connected with the RNA portion of the molecule. Gierer and Schramm (55) showed that after a complete removal of protein, RNA was itself infectious. Also, they showed that TMV protein could be changed chemically without affecting the activity and generic properties of the virus; and recently, parts of the protein complement of the nucleoprotein particle have been removed without destroying viral activity. This report went further in indicating that naturally occurring chemicals other than RNA had no viral activity.

The role of RNA can be clarified further by the work of Fraenkel-Conrat (46). This investigation was directed toward the production of "hybrid" viruses constituted from TMV protein with HR (Holmes ribgrass) infectious nucleic acid. Immunologically, the hybrid produced resembled TMV (the virus which supplied the protein coat); whereas its symptoms in the plant were similar to those of the HR strain.

Thus, the six criteria of Jacobs (70) for determining whether or not a substance can be considered a growth regulator would seem to apply to RNA in relation to cellular metabolism.

Soluble nucleotides: In a strict sense, nucleotides are N-glucoside phosphates containing a purine or pyrimidine base, with phosphoric acid esterified to one of the hydroxyl group of the sugars (5).

The acid-soluble nucleotides comprise one of the most active, varied, and versatile groups of compounds in nature. They are a part of the phases of carbohydrate, lipid, protein, and nucleic acid metabolism. Nucleotides are the pool of energy, as well as the naturally occurring medium, which is responsible for the transfer of energy. Many processes, e.g., oxidation, reduction, and epimerization, take place only when the substrate has been activated by combination with nucleotides. They play a vital role as a precursor of polymers of nucleic acid.

Nucleotides with which the substrate combine may involve specificities of great significance, e.g., coenzymes of cytosine combine with alcohols; uracil with aldehydes (sugars); and adenylic acid with amino acids, acetic acid, and sulphuric acid (67).

From the work of Cherry et al. (24, 25) on the effect of X-irradiation on corn seed and on acid-soluble nucleotides of different corn inbreds and hybrids, it is evident that changes in soluble nucleotides occurred in a manner roughly parallel to growth. The changes may also be correlated with vigor of the plant. Hybrids generally had a higher quantity of nucleotides in their embryonic axis than inbred lines. Motirmani (94) studied total and various cytoplasmic fractions and stated that concentration of soluble nucleotides increased considerably when the young corn plants were potassium deficient.

Since the RNA portion of virus seems to be responsible for the infectivity, a great deal of work has been done on the chemical composition of the RNA polymers. Reddi and Knight (113) found upon

analysis of the residual material after ribonuclease digestion that the rubonuclease-resistant residue of all five strains of TMV appeared to have the same composition. The residues were rich in purines and poor in pyrimidines and averaged about six nucleotides in composition. Cooper and Loring (35) examined the purine and pyrimidine composition of TMV particles and did not find any significant differences between the ordinary strain and Holmes' masked strain. De Fremery and Knight (38) studied three strains of bushy-stunt virus and found that all of them were similar in nucleotide composition. Also, Hart (66) stated the importance of the distribution of purine and pyrimidine bases in the nucleic acid of TMV as a tool to identify ribonucleic acids. However, he emphasized that hepta-nucleotide-like fragments after partial digestion of nucleic acid with ribonuclease would be more characteristic of the molecules from which they were obtained.

Carbohydrates

Disturbances in carbohydrate metabolism have been reported by several workers (42, 107, 128). Different reasons have been suggested for drastic changes in carbohydrate metabolism. Virus infection reduced chloroplasts and chlorophyll per plastid as the result of increased chlorophyllase. Peterson (109) studied three types of mosaic viruses and found the results which supported the above statement. The above reasoning might be true for the mosaic type of virus diseases, which is accompanied by decrease in total carbohydrates (17). Yet, with the virus which causes yellow disease, there was an increase in total carbohydrates (42). Rosa (115) showed an accumulation of carbohydrates

in total leaves infected with western-yellow-blight. True et al. (127) also reported an accumulation of carbohydrates in spinach leaves infected with spinach-blight virus. Campbell (21) indicated a higher percentage of sugars, as well as starch, in the aboveground portion of potato plants infected with potato leaf-roll virus.

Several suggestions have been made to account for the accumulation of carbohydrates in viral-infected material; for example, Woods (138) proposed that oxidizing enzymes inhibit takadiastase and maltdiastase by the phenomenon of oxidative destruction, which in turn allowed carbohydrates to accumulate. Along the same line, Grieve (64) indicated that the activity of hydrolytic enzymes was reduced as the result of viral infection.

True et al. (127) stated that accumulation of carbohydrates did not result from inability of the diseased plants to synthesize protein, but that carbohydrates accumulated because of slower growth of the diseased plants, with consequent diminished use of manufactured food. Another suggestion has been made that accumulation of carbohydrates resulted from lessened translocation caused by either necrosis of the phloem, or by changes in permeability of protoplasm.

Decreased amount of total sugars has been reported in the mosaic virus-infected plants and in virus-infected grape leaves. Whitehead (135) noted an increase in the amount of total sugars in the leaves of leaf-roll-infected plants and correlated this increase with the excess production of carbon dioxide.

True et al. (127) reported an increase in the amount of total sugars in the leaves of virus-infected spinach. True and Hawians (128), Shibata and Panaanelli, as reported by Wynd (139) found an increase of sucrose in blight-infected spinach plants, leaf-roll-infected mulberry leaves, and leaf-roll-infected grapes, respectively. On the other hand, Brewer et al. (17) reported a decrease in amount of sucrose in mosaic-infected tomato plants.

Dunlap (43) reported that mosaic-infected tobacco leaves contained only half as much reducing sugars as normal leaves. Decrease in amount of reducing sugars was also found associated with blight-infected tomato plants by Rosa (115), who reported an increase in the amount of reducing sugars in the leaves of blighted tomato plants; by Marcel and Catayee (84), who found more reducing sugars in plants infected with "court-noues" virus than those of healthy grape vines; and by Orlob and Arny (99), who stated that starch and soluble carbohydrates, especially reducing sugars, accumulated in barley-yellow-dwarf-infected plants.

Nitrogen constituents

Total nitrogen: True et al. (127) reported a lower percentage of total nitrogen in spinach leaves infected with spinach-blight. Rosa (115) stated that total nitrogen was higher in the leaves but was lower in other parts of certain plants infected with virus. Brewer et al. (17) postulated that in nearly all the cases of plants showing mosaic symptoms there was a small decrease in total nitrogen. Dunlap (42) gave a contradictory picture. He found that mosaic-like viral diseases were accompanied by an increase in total nitrogen, and yellow

types of viral diseases by a reduction in total nitrogen. Orlob and Arny (99) reported that barley infected with a virus known as yellow-dwarf caused a reduction in total nitrogen of leaves. Also Grigsby (65) analyzed total nitrogen in the leaves of red raspberry plants infected with a mosaic virus and found less total nitrogen than in healthy leaves.

Three reasons have been postulated for variations in the amount of total nitrogen. First, Rosa (115) believed that nitrogen depletion resulted from inability of the plant to absorb and translocate nitrogenous compound from the soil. Suzuki, as reviewed by Wynd (139), based his argument primarily on the fact that additions of nitrogenous fertilizers to the soil did not overcome the symptoms of nitrogen starvation of mulberry trees infected with leaf-roll virus. Second, accumulation of carbohydrates without a corresponding change in total nitrogen would lower the percentage of nitrogen, giving a dilution effect, an idea suggested by Wynd (139). Third, the lower percentage of total nitrogen may result from denitrification in some tissues, so that part of the nitrogen may be lost either as elementary nitrogen or in the form of ammonia (127).

Soluble protein: Martin et al. (86) indicated that total protein content of mosaic-infected tobacco plants seemed to have undergone little if any change. This work suggested that viral protein is synthesized at the expense of normal protein, but not necessarily directly from it. With the common mosaic type of virus, trypsin-resistant protein was regarded as a viral protein which existed in smaller proportion than

has been previously supposed. The amount of resistant protein was found to be greater in a susceptible variety of tobacco than in a variety generally considered non-susceptible. There is no proof at present time that the yellow mosaic virus is resistant to trypsin (86).

Wildman et al. (136) and Takahashi and Ishii (126) indicated that infection of tobacco with TMV may be accompanied by a concurrent decrease in the concentration of normal protein of leaves. Recently, Bawden and Kleczkowski (10) showed that concentration of normal protein was not affected by virus infection in all cases. A significant finding which may indicate the reasons for this variation can be found in a report by Meneghini and Delwiche (88). They showed that variation in the concentration of normal protein, resulting from virus infection, occurred because of competition between the viral protein and normal protein for precursors present in a metabolic pool, rather than for utilization of previously formed soluble proteins.

Amino acids: Commoner and Nehari (32) stated that there were transitory deficiencies in certain individual amino acids and amides during the period of rapid viral multiplication. Concentration of asparagine, glutamine, aspartic acid, glutamic acid, and serine was lowered as the result of infection in comparison to TMV-infected tobacco leaf discs and non-infected discs. Also Porter (110) studied free amino acids and amides of intact tobacco plants after inoculation with TMV and reported a net increase in serine, glutamine, and asparagine during the early period of infection.

A study was made by Allison (1) of a possible relation between glutamic acid and glutamine in potato tubers, on different varieties infected with leaf-roll virus. Diseased tissues had two to three times the glutamine concentration of healthy tissues; whereas glutamic acid differences were much smaller. In this same study tyrosine and tryptophan, however, showed no consistent differences between diseased and non-diseased tissues. This is in disagreement with previously reported work by Andreae and Thompson (2), who suggested that leaf-roll virus molecules might draw especially heavily on tryptophan and tyrosine for their synthesis, or divert them into the production of other substances. Also, an alternative pathway might be activated, such as conversion of tyrosine to scopoletin--a substance found in abnormal amounts in leaf-roll-infected plants (40). Perdrizet (107, 108) reported considerable changes in the metabolism of serine, proline, and aspartic acid in leaf-roll virus-infected potato plants. Filippo (52) found a decrease in glycine, lysine, histidine, leucine, and isoleucine content of virus-infected potato tubers.

After a careful investigation of free amino acid and amide content of tomato plants infected with tomato-spotted-wilt and healthy plants, Selman et al. (118) suggested that accumulation of NH_2 amide compounds might interfere with virus synthesis. Increases in glutamic acid, asparagine, glycine, and serine in the inoculated leaves were found.

Bailova-Yankulova (6) also found an increase in tryptophan and tyrosine in potato tubers. Magdoff et al. (82) were in agreement with Bailova-Yankulova when they studied the content of free amino acid of southern bean mosaic virus.

Lalorya and Govindjee (78) showed the absence of ninhydrin-reacting substances in the water extracts of leaves infected with TMV and tobacco-curl viruses. Furthermore, the water extracts of diseased leaves revealed the presence of two new bands corresponding to asparagine and histidine-lysine. The absence of threonine and glycine was established.

Diener and Dekker (40) isolated a ninhydrin-reacting substance, l-pipecolic acid, from western-X-diseased peach leaves. This compound was absent in extracts from mature, healthy leaves.

Govindjee et al. (57) reported the formation of asparagine and increase in contents of free amino acids, e.g., leucine, isoleucine, phenylalanine, and aspartic acid in virus-infected leaves of tobacco plants.

Ammonia: Commoner et al. (33) showed from their isotopic experiments with N^{15} labeled nutrient that the bulk of TMV nitrogen is derived from the free ammonia of the host tissue. Commoner and Dietz (30) reported a reduction in non-protein nitrogen in TMV-infected leaf discs, compared with non-infected discs during the time that TMV was being synthesized. Ammonia contributed most to this deficiency with a smaller reduction occurring in amide content. It was concluded from these experiments that virus is synthesized de novo from ammoniacal nitrogen and changes in other non-protein nitrogen constituents resulted from withdrawal of ammonia for TMV production.

Nitrate: Jodidi et al. (71) indicated that mosaic-infected spinach leaves contained only about half as much nitrate nitrogen as normal plants.

Carbohydrate versus nitrogen

There seems to be little doubt that there is a difference between the balance of carbohydrate to nitrogen in the mosaic type of virus, as compared with the yellow type of virus. Dunlap (43) found that infection by mosaic virus was accompanied by an increase in total nitrogen and a decrease in the total carbohydrates; whereas the opposite was true with leaves infected with a yellow type of virus.

Phosphorus

Arnon (3) discussed the important functions of phosphorus and its compounds in the plant and emphasized its important role in energy transfer and as a component of nucleotides and nucleic acids. The role of phosphorylated compounds was recognized in the synthesis of virus, either as a source of energy or as component parts in the synthesis of nucleic acids, proteins, and nucleoproteins. An understanding of utilization of such phosphorylated metabolites could aid in the elucidation of pathways of virus synthesis.

Holden and Tracey (68) found that local infection had negligible effects, but systemic infection decreased total phosphorus per plant and increased the total phosphorus as percentage of dry matter. Porter (110) also reported that Ryzhkov and Gorodskaya found that TMV infection decreased the total phosphorus in the leaves of growing tobacco plants.

Vayonis (130) studied changes in inorganic phosphorus, phosphate esters, and residual plus total phosphorus in tobacco leaves systemically invaded with TMV, at 12-hour intervals. Inorganic and total phosphorus were not affected soon after infection, but both decreased six days after inoculation.

Porter and Weinstein (111), using tobacco plants grown in nutrient solutions, observed that the non-nucleic acid and organic phosphorus of systemically invaded leaves were reduced to about 60 per cent of the control seven days after inoculation. Infection did not appear to affect materially the uptake of phosphorus from the nutrient medium. Therefore, it seems likely that infection was associated with a stimulated utilization of organic phosphorus compounds, such as phosphorylated sugars, nucleotides, etc., as an energy source or as a component part in the synthesis of more complex nucleotides.

Virus Infection in Relation to Respiration of Host

Plant viruses influence significantly the rate of respiration of the host. Changes in rate of respiration have been employed to diagnose the nature of the disease (11). For example, a decrease in photosynthesis and increase in respiration caused by mosaic type disease have produced the characteristic effect of lowering the carbohydrate/nitrogen ratio of the plant.

Takahashi (125), Belkengren (12), and Rohrbaugh et al. (114) reported a decrease in oxygen uptake of TMV-infected tobacco, western-X-infected cherry plants and tristeza-infected citrus plants, respectively.

On the other hand, O'Reilly (98) showed an increase in respiration of peach foliage infected with the western-X-disease virus. He also mentioned that the respiration of healthy leaves, determined on the fresh-weight basis, was greatest at the time the leaves were matured up to the terminal buds. Respiration in all leaves decreased from terminal to base leaves of the current season's growth. The increase

in oxygen uptake occurred when compared both on a fresh-weight and a total nitrogen basis. This disparity between the above observations may have resulted from the time and type of sampling. From the work of Wynd (140) and Owen (100, 101, 102, 103) it appeared that the respiratory rate of TMV-infected tobacco increased just after virus infection, but a decrease in rate of respiration followed until the rate was less than normal. Also, Orlob and Arny (99) reported that plants infected with the yellow-dwarf virus of barley showed a higher rate of respiration than normal plants during early stages of infection, but that the respiration of plants inoculated for 35-69 days was lower.

Recently, two hypotheses have been drawn up to account for the influence of viruses on respiration. Belkengren (12) postulated that viruses might damage the respiratory system. From his work, reversal of virus inhibition of oxygen uptake by 2,4-dinitrophenol seemed to indicate that either phosphate fixation or phosphate utilization was involved in the affected metabolism. Also, viruses might tie up or compete for nucleotides that are necessary for phosphate fixation or transfer.

The second suggestion was made by Millerd and Scott (91) after reviewing the respiratory changes of virus-infected plants. They stated that there are contradictory results about the effect of virus infection on respiratory rates of host plants. They suggested that as a general pattern, the respiration rate in diseased plants increases only during the first period after infection, and thereafter the rate of oxygen uptake is always less than that of normal leaves. They also

concluded from the study of Owen (103) that it was possible that viruses had different effects on the respiration mechanisms of different host plants.

Bawden (11) felt that a general statement could not be made, since only a few diseases had been studied, and most experiments had been insufficiently replicated and inadequately controlled. He also pointed out that there are other things to be considered in a generalized statement in addition to the time of sampling. This can be illustrated by the reports of Loebenstein (80), who has shown that absolute respiration values are dependent on leaf age, time of daily measurements, and preconditioning temperatures; of Yamaguchi (141), who has shown that the strain of virus and the physiological age of the infected tissue influence greatly the respiratory pattern; and of Burton (19), who has shown that the immediate availability of certain nitrogenous compounds plays a major role in the degree of increased respiration. To make it even more difficult to reconcile all facts in a generalized statement, seasonal influences on the patterns of respiration of infected tissues have been noted (101, 102). Also, in certain cases there was the absence of a consistent pattern in the respiratory changes, as was shown by Bell (13) with virus-infected Phaseolus vulgaris.

Virus Infection in Relation to Gibberellic Acid

Gibberellins, a group of growth promoters, have a profound effect on growth and development of plants. Effects of gibberellins on plant growth have been studied extensively, especially the modifications of plant growth and development. From these and similar studies, there is

considerable evidence to support the idea that gibberellins are phytohormones (120). However, at present, little is known of the mechanism of action of gibberellins.

Kutsky and Rawlins (77) and Limasset et al. (79) reported therapeutic uses of auxins--a generic group of compounds that includes some phytohormones--against tobacco mosaic virus and the X and Y viruses of tobacco, respectively. Also, Grieve (64) concluded from his study of tomato plants infected with spotted wilt to the bronzing stage that the hormone balance was disturbed. This disturbance resulted from either a destruction or depression of the production of growth substances or an inactivation of auxin during translocation to growing points. Since auxin is necessary for cell elongation, its activation in greater or lesser degrees in infected plants must be considered an important factor in relation to inhibition of growth (7).

Recently, Chessin (26) postulated an upset in the growth regulator balance of plants visibly stunted by viral infection. He pointed out that stunting was characteristic of many virus infections when this was accompanied by symptoms such as witches' broom or premature leaf abscission. In these instances, he suggested that gibberellins might be important. He further stated that gibberellic acid is normally involved in stem elongation in higher plants; and stunting as a result of virus infection, might be the result of a reduced concentration of gibberellic acid in the plant.

Maramorosch (83) and Pavillard (106) also showed a reduction in the amount of free auxins in virus-infected plants. In addition to a

decrease in auxins, Pavillard (106) showed that a precursor of auxin was present in a much lower concentration in diseased than in healthy potato tubers. These findings agree with the work of other investigators who have found that tryptophan was absent in leaf-roll-diseased potato tubers (2).

Elongation of the internodes has been reported as a most typical and striking effect of gibberellic acid treatment. Stowe and Yamaki (123), and Sachs (116) reviewed the visual symptoms in general. The effect of gibberellic acid on elongation may result in a change in morphology of the plant, in some cases from bushy to vinelike characteristics (137). Increase in length and chlorosis of leaves are also brought about by gibberellins (123).

Virus Infection in Relation to Thiouracil

Several investigators have reported that the processes involved in the production of viruses are dependent on the metabolism of the host cells. It has been postulated that certain inhibitors influence virus infectivity by altering the physiological processes of the host in such a way that synthesis of the virus is reduced or prevented (11).

From the work of Bawden and Kassanis (9) and Mercer et al. (89) it appeared that a uracil-demanding system of the host was concerned in nucleic acid and protein synthesis. TMV multiplied more readily in detached leaves floated in sucrose-phosphate buffer and kept in light than in leaves floated in water and kept in dark. Thiouracil, an analogue of uracil and a competitive inhibitor, had little effect on the amount of virus produced in leaves which were kept in dark;

whereas it inhibited multiplication of virus when applied to illuminated leaves cultured in nutrient solution. Commoner and Mercer (31) have shown that thiouracil inhibited biosynthesis of TMV, and the inhibitory effect was reversed by uracil. Their suggestion was that thiouracil blocked the synthesis of TMV by interfering with some aspects of uracil incorporation into the polymer RNA.

Whereas thiouracil proved to be an effective inhibitor of TMV synthesis, it had little inhibitory effect on the synthesis of cucumber mosaic virus. Since the latter also has uracil in the make-up of the RNA portion, the different effect would seem to indicate an action involving host metabolism (15). Francki and Mathews (51) found that thiouracil suppressed multiplication of turnip-yellow mosaic virus nucleoprotein but caused a measurable increase in the production of virus protein shells which were devoid of RNA. They further stated that thiouracil is not incorporated into the RNA of the complete virus. Thus, thiouracil seems to interfere with RNA synthesis before incorporation into the particle.

From experiments with 2-thiouracil-S^{35} and varmorr 48 tobacco, Porter and Weinstein (112) suggested that thiouracil interferes with virus multiplication by affecting the metabolism of the host by incorporating in the synthetic mechanism responsible for formation of virus nucleoprotein. Francki (50) and Gordon (56) also supported the statement of Porter and Weinstein that uracil is utilized in the production of viral RNA.

Kurtzman et al. (76) found that thiouracil in concentrations of 10 to 100 ppm was effective in inhibiting both TMV multiplication

and cellular activity of host tissue. Francki (49) inhibited multiplication of tobacco necrosis virus in both French bean and tobacco plants. Further, he noticed similar injury symptoms in both virus-infected and healthy host plants.

Porter and Weinstein (111), in observing biochemical changes induced by thiouracil in cucumber mosaic virus-infected and non-infected tobacco plants, stated that the severity of symptoms was increased by thiouracil treatment. Chlorosis, cupping, and strapping of leaves were noticed as thiouracil toxicity symptoms. Both infected and non-infected plants grown in nutrient solutions containing 3 and 5 ppm of thiouracil exhibited reduced dry weight of leaf tissue and lower levels of total amines, ammoniacal nitrogen, and organic and inorganic phosphorus in the leaves.

MATERIALS AND METHODS

To investigate possible changes in the physiology of citrus as a result of viral invasion, 'Key' lime was selected as the host plant, and a severe strain of tristeza (T_3) was chosen as the inoculum. The response of 'Key' lime to infection by this virus is rather rapid and accounts for its use as an indicator plant to detect the presence of tristeza in other citrus materials.

Seeds of 'Key' lime fruit obtained from a local wholesale grocery in Gainesville, Florida were germinated in an incubator maintained at a temperature of 32°C. After careful screening, normal, healthy, true-to-type seedlings were transplanted to 4-inch pots containing a mixture of soil, peat, and perlite in a ratio of 2:1:1, respectively. The plants were kept in a greenhouse for approximately six months. Then, after another careful selection was made to discard the off-type seedlings, the plants were transferred to a climate-controlled chamber maintained at 20°C and on a 12-hour day and 12-hour night regime. Light intensity of the day was approximately 1000 ft-c. supplied by "daylight" fluorescent lamps.

Ten days after the plants were transferred to the climate-controlled chambers and just prior to chemical and viral treatment, data on the height of the plant and total number of leaves per plant were recorded for use as an analysis of the uniformity of

the population. This study indicated a fairly constant number of leaves throughout the seedling population but exhibited a plus or minus 1.5 cm in the height of the plants.

Experiment I

Experiment I included three variables, virus inoculated plants, gibberellic acid (10^{-3} M) treated plants, and thiouracil (3 ppm) treated plants, and all possible combinations of these agents (Table 1). Each of the eight treatments was replicated twice with six plants per replication. A total of 96 plants was used for the treatments which were arranged in a split-plot design.

Inoculation

The 'Key' lime plants were infected with an inoculum of tristeza (T_3) obtained from Dr. T. J. Grant. The procedure followed that described by Grant and Higgins (62). Infected leaf pieces were taken from a sweet orange seedling that previously had been inoculated by means of leaf pieces obtained from other seedlings which had the T_3 (severe strain) virus transmitted to them by aphids. Two infected leaf pieces were placed under the bark at a uniform height of about three inches above the soil surface. The top edge of the leaf was left exposed as an indicator of successful union and virus transmission. If the leaf piece remained alive, a successful union was considered to have taken place. After eight days the plants were cut back to a single main stem about ten inches high. As lateral branches formed, all were pruned from the seedlings except three well-spaced ones above the graft.

TABLE 1

OUTLINE OF THE TREATMENTS OF 'KEY' LIME SEEDLINGS WITH VIRUS, GIBBERELLIC ACID (10^{-3}M), AND THIOURACIL (3 ppm) AND COMBINATIONS OF THESE FACTORS

Plant material	Code No.	Chemical treatment
Healthy plants	1	None (control)
	2	Gibberellic acid
	4	Thiouracil
	6	Gibberellic acid plus Thiouracil
Plants infected with tristeza	0	None
	3	Gibberellic acid
	5	Thiouracil
	7	Gibberellic acid plus Thiouracil

Chemical treatment

Virus-infected and non-infected plants of 'Key' lime were sprayed with gibberellic acid (10^{-3} M), and 2-thiouracil (3 ppm) as outlined in Table 1. The first application of gibberellic acid and thiouracil was two days before the plants were infected. After the initial spraying, gibberellic acid and 2-thiouracil were applied three additional times at intervals of seven days.

Sampling techniques

A random method of composite sampling was used in which leaves of uniform age, as judged by size and position on the stem, were collected for the various analyses at interval of seven days from inoculation. Freshly harvested leaves were used for determinations of nucleotides, amino acids, oxygen uptake, total nitrogen, total sugars, and reducing sugars.

Dried samples of leaves were used for total phosphorus, potassium, and calcium.

Growth measurements and symptomatic observations

Treated and control plants were observed closely for changes in growth and development. The length of each lateral was recorded weekly as an index of growth rate. Symptomatic appearances of vein clearing, cupping, and yellowing of the leaves were noted when they occurred. Transmitted light was used to differentiate the degree of vein clearing once it had been detected.

Determination of oxygen uptake

For oxygen measurements, Warburg constant volume respirometers were used. After a preliminary study of the conditions needed for reliable oxygen uptake measurements, the following procedure was adopted. Warburg flasks were prepared for the tissue by placing 10 per cent KOH-soaked filter paper in the center well and a filter paper disc saturated with distilled water (0.2 ml) in the main compartment of flask. Leaves were collected at 10:00 p.m. each time of sampling. Ten leaf discs (45 to 60 mg) were prepared with the help of sharp hole puncher and placed in each flask. The chambers were equilibrated for two hours at 28°C. During the entire time of measuring the gaseous exchange pattern (one hour), the apparatus was kept at constant temperature of 28°C plus or minus 0.05°C. The changes in gas volume were calculated according to the methods explained by Umbriet (129).

Determination of total nitrogen

Total nitrogen was determined by using four leaf discs (16 to 23 mg) from the leaves which were previously used for oxygen uptake measurements. Total nitrogen was determined by the micro method of nesslerization described by Johnson (72). In the present experiments nesslerization and micro-kjeldahl procedures were also compared by analyzing a standard sample. The difference between the two methods was less than 1.5 per cent and a greater precision of nitrogen determination was obtained by the nesslerization procedure when 20.0 mg of fresh leaf tissue was used as a sample.

Determination of total and reducing sugars

Sugars were determined by the colorimetric methods as outlined by Nelson (95). Briefly, the procedure was as follows: Fresh samples of leaves (0.5 to 0.8 g) were quick frozen inside a container placed in a dry ice-acetone bath. After two hours, the frozen tissues were ground with the aid of a glass rod with sharp edges which facilitated the grinding. After grinding, the frozen sample was transferred directly to 80 per cent ethyl alcohol in a thimble in a soxhlet apparatus. After eight hours of continuous extraction, the extractant was removed from the soxhlet chamber and alcohol evaporated from the extract with the use of a water bath maintained at 65°C. The gummy residue was dissolved in a small amount of water before it was cleared by lead acetate and centrifugation. The cleared extract was used for either reducing or total alcohol-soluble sugars determinations. For the determination of total sugars, enzymatic hydrolysis by invertase (1 per cent) was used with a ten-hour incubation period at 30°C.

Determination of phosphorus, potassium, and calcium

For the determination of phosphorus, potassium, and calcium, fresh leaves were dried at 70°C in a forced-draught oven for approximately ten hours, with the material spread out as thinly as possible. An attempt was made to treat all samples identically. The samples, dried to constant weights, were ground to a fine powder with the aid of a mortar and pestle.

To obtain a digested plant sample, a combination of standard dry and wet ashing procedures was followed.

Total phosphorus was determined by a colorimetric procedure developed by Cavell (23) which made use of elon and ammonium molybdate as the chromophoric developing agents.

Calcium and potassium were determined by emission spectroscopy. The Beckman DU flame spectrophotometer was used at a wave length of of 622 millimicron to detect calcium and a wave length of 768 millimicron to detect potassium.

Determination of transmissibility of virus from plants under observation

A study of the symptomatology of various treatments indicated that a change had occurred. To determine whether or not this change was temporary or stable and could be transferred, plants of 'Key' lime were grafted with the leaf pieces from each of the treatments. The same procedure was repeated again after symptoms had appeared on these plants.

Experiment II

Since the preliminary test indicated that the gibberellic acid greatly modified the influence of tristeza on citrus, a second experiment was attempted. Instead of applying gibberellic acid directly to the host, plant tissue containing the virus was treated and then grafted on to a receptor plant. Healthy and infected 'Key' lime leaves were soaked in solution with or without addendum for eight hours at 45°F. The addendum was either 10^{-3}, 10^{-4}, or 10^{-5} M GA. After soaking, the

leaves were rinsed with distilled water and used as the source of inoculum. Symptomatic patterns were closely observed as they developed.

Experiment III

Because of time limitations all biochemical measurements could not be carried out at the same time. Therefore, a third series of tests was conducted to determine changes in soluble nucleotides and amino acids, as a result of tristeza infection of 'Key' lime. In addition to these determinations, measurement of oxygen uptake was again followed from inoculation until visible symptoms appeared on the plants. Conditions for these tests were somewhat different from the first experiment; the plants were 18 months old, and they were kept in a greenhouse for the duration of the tests. However, the source of inoculum, method of inoculation, type of symptomatic observations, and determination of oxygen uptake were the same as in the first series of tests.

Examination of the free amino acid content of leaves

Fresh leaves (1.5 to 2 g) at a stage of vigorous growth were collected and immediately frozen by placing them in a container in dry ice-acetone bath. The frozen leaves were ground and then extracted with hot redistilled 80 per cent ethanol for ten minutes. After obtaining the filtrate, it was concentrated by drying under vacuum. The residue was treated to extraction by 2 ml of 80 per cent ethanol. Amino acids present in this extract were separated by paper chromatography.

Standard amino acids (10 lambda) and plant extract (20 lambda) were subjected to double dimensional paper chromatography. The solvents used were n-butanol:acetic acid:water (4:1:5 v/v) and phenol:water (5:1 v/v). The color reagent used was 0.2 per cent ninhydrin in ethanol. Unknowns were tentatively identified by comparing their Rf values to those of known amino acids. The quantity of any amino acid was determined visually by intensity of a chromophore and area of a spot.

Examination of soluble nucleotides present in the tissue

Soluble nucleotides were separated and identified by a modified procedure explained by Cherry et al. (24). The procedure used to separate the nucleotides from 'Key' lime leaves included paper and ion exchange chromatographic techniques and spectroscopic analysis of the components isolated.

Reagents: Perchloric acid . . . . . 0.6 N

Ammonium Formate . . . . . 1.6 N

Sodium Formate . . . . . 3.0 M

Formic acid . . . . . 4.0 M

Ion exchange resin-Dowex 1 X 8- 100-200 mesh

0.1 sodium phosphate buffer, pH 6.8; ammonium sulphate; n-propanol (100/60/2 v/v).

5'-Ribonucleotides (all from Pabst Laboratories):

Adenosine . . . .

Adenosine monophosphate . . (AMP)

Adenosine diphosphate . . . (ADP)

Adenosine triphosphate . . (ATP)

Guanosine . . . . . .

Guanosine monophosphate . . (GMP)

Guanosine diphosphate . . . (GDP)

Guanosine triphosphate . . (GTP)

Cytidine . . . . . .

Cytosine monophosphate . . (CMP)

Cytosine diphosphate . . . (CDP)

Cytosine triphosphate . . . (CTP)

Uridine . . . . . .

Uridine monophosphate . . . (UMP)

Uridine diphosphate . . . . (UDP)

Uridine triphosphate . . . (UTP)

Diphosphopyridine nucleotide (DPN)

Triphosphopyridine nucleotide (TPN)

Preparation of column: An ion exchange column 25 cm x 2.5 cm was prepared by using Dowex-1X8 100-200 mesh, anion exchange resin in the chloride form. In order to change the column from a chloride to a formate form, the resin bed was washed with 3 M sodium formate until chlorides were completely removed. After the column was saturated with the formate ion, it was washed with water to remove excess formate.

Preparation of standards: Standards of 5'-Ribonucleotides listed under "reagents" were prepared by dissolving 8 mg.in 2 ml of water. A known amount of the chemicals was used to standardize the separation and detection techniques.

Preparation of plant material for determinations of soluble nucleotides: In obtaining plant material for the determination of soluble nucleotides, care was taken each time to get a composite sample that was as uniform as possible. Both healthy and infected plants were sampled at 0, 7, 14, and 28 days after inoculation. Composite samples were made up of entire young growing apices that were decapitated from the plant just below the young expanding leaf that was 1 cm ± 2 mm long. Each sample was composed of enough growing buds to weigh 6-7 g. Immediately upon detaching the apices from the plant, they were quick-frozen by dropping them in a container placed in a dry ice-acetone bath. Grinding of the sample was done in the dry ice-acetone bath by pulverizing the frozen tissue to a fine powder with a plunger. Then, the ground sample was transferred while still frozen to cold 0.6 N perchloric acid and subjected to mixing and homogenization with Omni Mixer for four minutes. Care was taken to keep the temperature from rising above 3°C. After the grinding and mixing, the mixture was allowed to stand for three hours in an ice bath. Then the extract was filtered; the filtrate neutralized to pH 6 to 7 by adding 5 N potassium hydroxide; and the potassium chlorate precipitate removed by filtering. Throughout this last operation, the extractant was kept at temperature below 3°C.

Operation of the column: The nucleotides were separated by gradient elution from a Dowex resin 1X8 in the formate from which was subjected to a solvent gradient between water and 4 N formic acid followed by a gradient between 4 N formic acid and 1.6 N

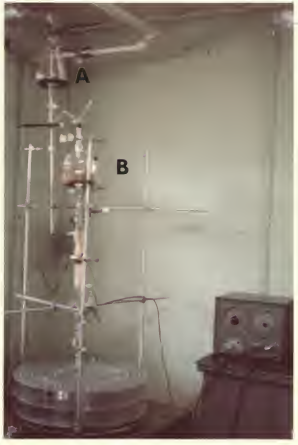

Figure 1. Apparatus used for gradient elution analysis.

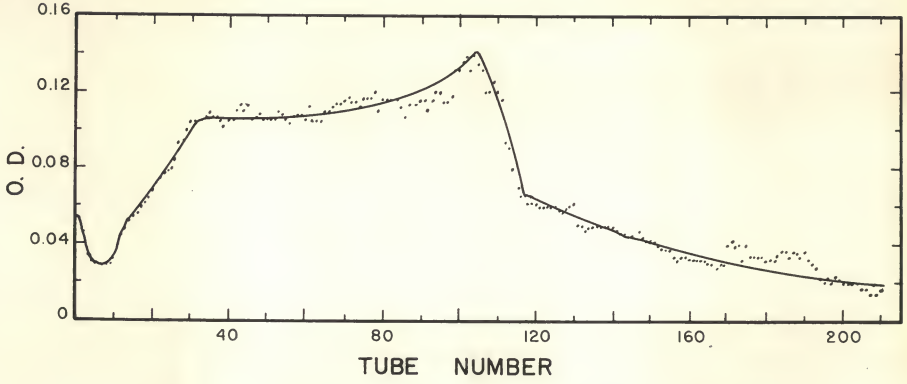

Figure 2. Pattern of the formic-formate gradient elution solvent from the ion-exchange column.

ammonium formate. Figure 1 illustrates the apparatus used. It consists of a 1 L reservoir (A), a 1 L mixing flask (B) and a resin column 25 cm x 2.5 cm in length. This was used in conjunction with a fraction collector which collected in volumes of 10 ml.

Before analyzing a plant sample the physical set-up was standardized as follows: A column was subjected to the solvent system but without the addendum on the column. Since formic acid has a weak absorbance at 260 millimicrons, the change in absorbance was followed as the column was developed. This pattern can be found in Figure 2. Notice that the vertical O.D. scale covers only 0 to 0.16. To obtain this pattern, one-tenth of a liter of distilled water was allowed to pass through the column before 4 N formic acid was allowed to pass from the top reservoir (A, in Fig. 1) into mixing flask (B, in Fig. 1) containing 1 L of water. Thus, a drop flowed from the column, a drop from the middle reservoir flowed into the column, and a drop from the top reservoir flowed into the middle flask. A crucial part of this set-up was to obtain instant mixing in the middle flask. After the formic acid had flowed from the top reservoir into the middle reservoir, the operation was continued until it had all passed into the middle reservoir. Throughout the entire operation, 10 ml fractions of the eluate were collected.

Screening fractions for nucleotides: Fractions of the eluate from the ion exchange column were scanned for possible nucleotides using a Beckman DU spectrophotometer at a wave length of 260 millimicrons. If absorbance indicated the possible presence of a nucleotide in any

fraction, continuous spectral curves of the sample at pH 2 and 7 were prepared with the aid of a Beckman, model DB, spectrophotometer. The aleviation of the interference from formic acid was achieved by lyophilizing the fraction which distilled the acid from the sample. Thus, the residue was redissolved in distilled water and the pH adjusted with 0.1 N HCl and NaOH and sodium diphosphate. The quantity of a nucleotide was determined by calculating the total area under the elution-pattern curve. Spectroscopic analysis was made on the fraction containing the greatest quantity of nucleotides.

Statistical Analyses

Data collected in Experiment I were analyzed statistically on the split-plot design (121). Significant differences in treatments and days after infection were calculated by means of new Duncan multiple range test (41).

EXPERIMENTAL RESULTS

These investigations were conducted to gain a better understanding of the influence of a virus on the physiology of a host. As an index of physiological changes, several observations were made, which will be reported from three major experiments.

Experiment I

The first series of tests was made concurrently on the same plants, except for the stability and transmissibility study. They were designed to determine the influence of tristeza on growth and symptomatic changes of 'Key' lime and to determine the effect of the virus on patterns of oxygen uptake, total nitrogen, alcohol-soluble sugars, phosphorus, potassium, and calcium. In addition to this, the influence of GA and thiouracil on non-infected and infected 'Key' lime was determined using the same measuring parameters.

Effect of tristeza, gibberellic acid, thiouracil, and interactions of these agents on growth and on symptomatic changes of 'Key' lime.

Length of lateral branches was taken as a criterion of growth and recorded at intervals of seven days from the beginning of the test until visual symptoms of viral infection were evident. Measurements were made on the laterals, since the main axis was pruned from the plants 20 cm above the point of the graft on the seventh day after inoculation. It

is evident from Table 2 and Figure 3 that tristeza had a suppressing effect on growth. This was apparent by the twenty-first day after inoculation. By the forty-second day after infection, the total average length of lateral branches was decreased 61 per cent by the virus, as compared to the control.

Gibberellic acid alone stimulated the growth of the lateral branches. There was an increase in length of lateral branches up to 95 per cent on the twenty-eighth day. It is interesting to note that during the first 21 days there was very little difference between the GA-treated plants and the controls, all of the increase taking place in the fourth week.

When either thiouracil or virus was tested on the same plants as GA, the growth was very similar to that of plants treated just with GA. It would seem that GA can reverse the suppression of stem elongation caused by the virus, and that thiouracil has little or no effect on this reaction.

Also, from Table 2 and Figure 3, there was not a significant difference in the length of lateral branches of thiouracil-treated and non-treated plants. Tristeza in combination with thiouracil suppressed the growth of 'Key' lime and showed the same pattern of suppression of growth as the virus alone. Thus, it would seem that thiouracil had very little effect on this aspect of the action of tristeza under the conditions of these tests.

In addition to measurements on length of lateral branches, observations were made periodically for vein clearing, cupping of the leaves, abscission of young leaves and tip necrosis. Photographs of representative plants from each treatment have been included in this report to

TABLE 2

THE INFLUENCE OF TRISTEZA, GIBBERELLIC ACID, THIOURACIL, ALONE AND IN VARIOUS COMBINATIONS, ON THE GROWTH OF LATERALS OF 'KEY' LIME

Days from inoculation	Length of lateral branches (cm)							
	Without Virus				With Virus			
	Control	GA	Thio	GA plus Thio	Control	GA	Thio	GA plus Thio
0	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-
14	0.82	2.98	-	0.72	-	-	0.33	-
21	4.78	4.13	4.48	3.99	2.68	4.35	3.55	4.83
28	7.83	15.31	9.11	14.01	4.08	13.83	5.18	14.66
35	10.23	-	9.66	-	4.09	-	5.32	-
42	11.65	-	12.30	-	4.57	-	5.70	-

GA Gibberellic acid
Thio Thiouracil

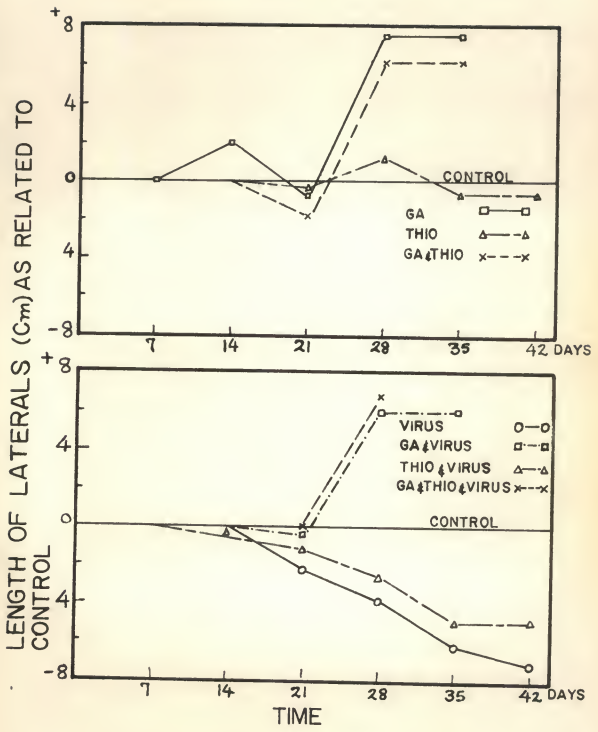

Figure 3. Effect of tristeza, gibberellic acid (GA) and thiouracil (Thio), and interactions of these agents on length of laterals (Cm) of 'Key' lime.

illustrate some of the symptomatic changes that were noted. The condition of the plants three days after inoculation with the virus and the first application of GA and thiouracil and four days before the plants were decapitated 20 cm above the point of grafting is shown in Figure 4. Notice all treatments which included GA were already showing increased stem elongation by the third day.

Photographs were also made of certain plants forty-two days after the tests were initiated. In this case, a representative plant from a treatment was compared to the control. As can be seen in Figure 5, the infection of 'Key' lime with tristeza caused a suppression of growth and a variety of other symptomatic changes which were in agreement with previously published observations (58, 59, 62). Briefly, these symptomatic changes included vein clearing, cupping of the leaves, abscission of young expanding leaves and tip necrosis. Vein clearing became apparent on the plants between the twenty-eighth and thirty-fifth day after inoculation. Abscission of the young leaves and tip necrosis was not apparent until about the forty-second day after inoculation.

Gibberellic acid accelerated the elongation of lateral branches both of tristeza-infected and non-infected plants. This can be seen in Figures 6 and 7. Contrary to what may be expected, GA also hastened the onset of symptoms normally associated with tristeza infection of 'Key' lime; namely, abscission of young leaves, vein clearing, leaf cupping, and tip necrosis. These symptomatic changes were apparent within thirty-five days after inoculation.

Thiouracil had little visible influence on 'Key' lime plants, as can be seen in Figure 8. However, when thiouracil treatments were

Figure 4. Representative plants from the various treatments, three days after infection and chemical application, of a test of the influence of tristeza, gibberellic acid, thiouracil, and interactions of these agents on 'Key' lime. (0 = virus, 1 = control, 2 = gibberellic acid, 3 = GA-plus-virus, 4 = thiouracil, 5 = thiouracil-plus-virus, 6 = GA-plus-thiouracil, 7 = GA-plus-thiouracil-plus-virus.)

Figure 5. Plant infected with tristeza, compared to a control plant on the forty-second day after inoculation. (Left = virus and right = control.)

Figure 6. Comparison between a gibberellic acid-treated plant and the control on the forty-second day after the test was initiated. (Left = GA and right = control.)

Figure 7. Comparison between a gibberellic acid-plus-virus-treated plant and a control plant on forty-second day after treatments began. (Left = GA-plus-virus and right = control.)

Figure 8. Representative plant treated with thiouracil, compared to a non-treated plant on the forty-second day after the tests were initiated. (Left = thiouracil and right = control.)

combined with tristeza inoculations, it lessened the period for the appearance of visible symptoms and increased the severity of the symptoms. The appearance of the plants forty-two days after treatment can be seen in Figure 9. Notice the severity of the stunting of growth.

The plants treated with GA and thiouracil were very similar to the plants treated with only GA. (compare Figures 10 and 6). Also, plants treated with GA, thiouracil, and tristeza were very similar to plants treated with GA and tristeza (Figs. 11 and 7). It would seem that the influence of GA was so great on the plants that the action of thiouracil was masked. However, GA was not able to mask the influence of tristeza on 'Key' lime, except for reversing the "stunting" influence of the virus. In fact, the other symptomatic changes were hastened and intensified.

Effect of tristeza, gibberellic acid, thiouracil and interactions of these agents on several chemical constituents of 'Key' lime

To examine further the influence of tristeza on the physiology of 'Key' lime and to investigate whether gibberellic acid or thiouracil modify the reactions between the virus and host, determinations were made of changes in the leaves of total nitrogen, phosphorus, gaseous oxygen exchange, potassium, calcium, and alcohol-soluble sugars.

Determinations of total nitrogen: The leaf content of nitrogen was determined periodically from the date of inoculation until visual symptoms of viral infection appeared. Viral invasion did not drastically

Figure 9. Comparison between a plant treated with thiouracil and tristeza and a non-treated plant on the forty-second day of the test. (Left = thiouracil-plus-virus and right = control.)

Figure 10. Illustration of the influence of gibberellic acid and thiouracil on the growth of 'Key' lime plants on the forty-second day after the first treatment. (Left = GA-plus-thiouracil and right = control.)

Figure 11. Comparison between a plant treated with gibberellic acid, thiouracil, and tristeza and a non-treated plant on the forty-second day after the test was initiated. (Left = GA-plus-thiouracil-plus-virus and right = control.)

change the total nitrogen content of the tissue.(Table 3 and Fig. 12). There was a slight decrease in the total nitrogen content of the leaves on every sampling date after inoculation. The greatest difference between infected and non-infected leaves occurred on the twenty-eighth day. A different type of tissue was sampled on the thirty-fifth and forty-second days; namely, young expanding leaves on the lateral that were initiated and grew after the plants were inoculated.

From a further comparison of the data in Table 3 and Figure 12 it can be ascertained that GA had a greater depressing influence on the total content of nitrogen than either the virus or thiouracil, and the most pronounced difference between the control and GA-sprayed plants was apparent on the twenty-eighth day. It is interesting to note that the depressing influence of GA and tristeza on total nitrogen was not additive.

Determinations of total phosphorus: As can be seen from the data in Table 4, and as shown graphically in Figure 13, tristeza alone had the greatest influence on total phosphorus. An increase in the phosphorus content of the leaves was evident on the seventh and fourteenth day after inoculation, and a decrease on the twenty-eighth, thirty-fifth, and forty-second days. The increase in phosphorus on the seventh day amounted to more than twice that of the control, and the decrease on the forty-second day was such that there was only one-third as much present as in the control.

In contrast to the effects of the virus on the phosphorus content of leaves, the influence of GA or thiouracil or a combination of the

TABLE 3

THE INFLUENCE OF TRISTEZA, GIBBERELLIC ACID, AND THIOURACIL, ALONE AND IN VARIOUS COMBINATIONS, ON CHANGES IN TOTAL NITROGEN OF 'KEY' LIME LEAVES (PER CENT OF FRESH WEIGHT)

Days after initiation of treatments	Source of Material	Per cent total nitrogen, based on fresh weight, in leaves of 'Key' lime							
		Without Virus				With Virus			
		Control	GA	Thio	GA plus Thio	Control	GA	Thio	GA plus Thio
0	A*	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49
7	A	0.68	0.64	0.79	0.68	0.58	0.76	0.70	0.72
14	A	0.96	0.79	0.90	0.92	0.91	0.78	0.89	1.01
21	A	1.18	1.03	1.14	1.00	1.15	1.00	1.06	0.94
28	A	1.25	0.85	1.35	1.07	1.05	1.00	1.07	1.13
35	B**	0.54	0.44	0.59	0.49	0.45	0.43	0.49	0.43
42	B	0.46	--	0.48	--	0.32	--	0.29	--

* A - Young fully expanded leaves present on the main axis of the seedling at the time of inoculation.

** B - Young expanding leaves present on lateral branches which were initiated after inoculation.

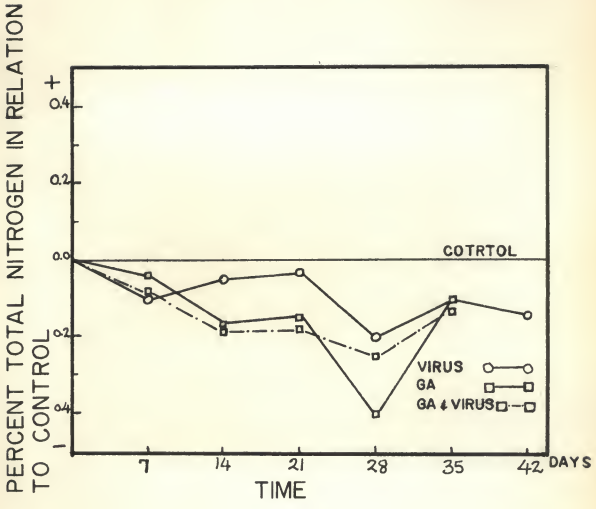

Figure 12. The influence of tristeza and gibberellic acid (GA) and the interaction of these two agents on the changes in the total nitrogen content of 'Key' lime leaves.

TABLE 4

THE INFLUENCE OF TRISTEZA, GIBBERELLIC ACID, AND THIOURACIL, ALONE AND IN VARIOUS COMBINATIONS, ON CHANGES IN TOTAL PHOSPHORUS OF 'KEY' LIME LEAVES

Days after initiation of treatments	Source of Material	Per cent total phosphorus, based on dry weight, in leaves of 'Key' lime							
		Without Virus				With Virus			
		Control	GA	Thio	GA plus Thio	Control	GA	Thio	GA plus Thio
0	A*	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
7	A	0.14	0.15	0.18	0.19	0.32	0.21	0.18	0.29
14	A	0.17	0.21	0.18	0.17	0.23	0.20	0.23	0.25
21	A	0.20	0.25	0.24	0.24	0.21	0.20	0.28	0.20
28	A	0.26	0.28	0.20	0.25	0.23	0.21	0.22	0.27
35	B**	0.26	0.12	0.15	0.13	0.11	0.14	0.11	0.16
42	B	0.26	--	0.13	--	0.08	--	0.10	--

* A - Young fully expanded leaves present on the main axis of the seedling at the time of inoculation.

** B - Young expanding leaves present on lateral branches which were initiated after inoculation.

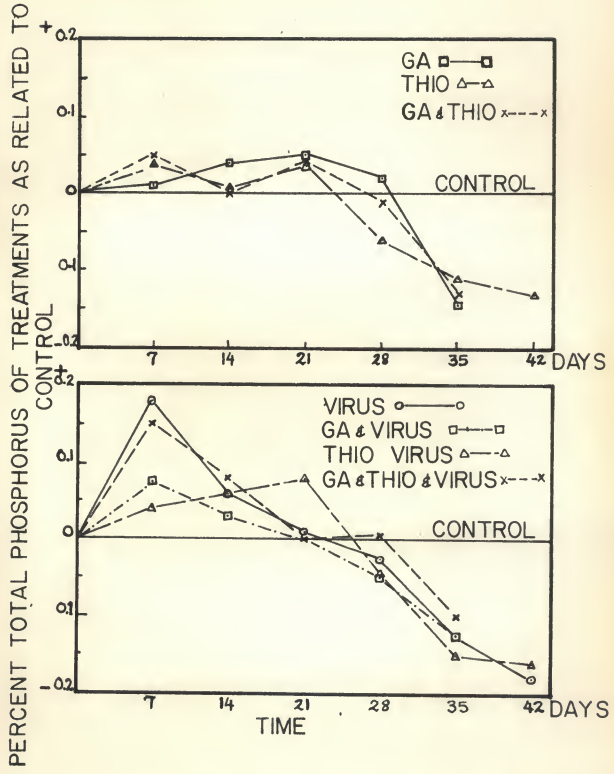

Figure 13. Effects of tristeza, gibberellic acid (GA), thiouracil (Thio), and interactions of these agents on the total phosphorus content of 'Key' lime.

two (Fig. 13) was much less. There may have been a slight increase in tissue phosphorus by the chemicals during the earlier sampling. However, statistically, the only increase that was significant was the GA-treated plants on the twenty-eighth day. Both GA and thiouracil, and a combination of these agents, significantly decreased the level of phosphorus in the leaves on the thirty-fifth and forty-second sampling dates.

When either GA or thiouracil was applied to the plants in conjunction with the virus, it altered the pattern of total tissue phosphorus (Fig. 13). The greatest alteration in the pattern occurred during the early viral infection stage, a depression of the increase in total phosphorus caused by the virus. When both GA and thiouracil were applied on the virus-treated plants, however, the pattern of tissue phosphorus was very similar to that of the plants treated only with the virus.

Determination of oxygen uptake: Tristeza increased the uptake of oxygen significantly during the early period of infection. As shown in Table 5 and Figure 14, the consumption of oxygen by the viral-infected leaves on the seventh day was almost twice as much as the non-infected leaves. The consumption of oxygen by infected tissue decreased on the fourteenth and twenty-first sampling dates, but it was still slightly higher than the control. By the thirty-fifth and forty-second sampling dates the uptake of oxygen by the infected tissue was significantly lower than that of non-infected tissue. Thus, it would seem that during the early stages of infection an increased oxygen uptake occurs, while in the later stages, just prior to and during the occurrence of visible symptoms, the oxygen consumption of infected tissue decreased.

TABLE 5

THE INFLUENCE OF TRISTEZA, GIBBERELLIC ACID, AND THIOURACIL, ALONE AND IN VARIOUS COMBINATIONS, ON CHANGES IN OXYGEN UPTAKE OF 'KEY' LIME TISSUE

Days after initiation of treatments	Source of Sample	Oxygen uptake (ul/g F.W.) of 'Key' lime leaf discs							
		Without Virus				With Virus			
		Control	GA	Thio	GA plus Thio	Control	GA	Thio	GA plus Thio
0	A*	19.30	19.30	19.30	19.30	19.30	19.30	19.30	19.30
7	A	22.32	22.04	39.30	49.38	41.96	20.17	32.39	30.39
14	A	31.70	32.46	41.24	33.01	38.50	34.23	33.10	36.01
21	A	33.70	37.65	39.31	34.48	36.58	37.43	35.57	35.08
28	A	25.29	40.34	39.52	41.43	25.04	41.92	37.90	38.86
35	B**	35.82	43.93	47.57	46.93	31.01	39.83	42.06	43.50
42	B	44.39	--	45.23	--	35.42	--	32.51	--

* A - Young fully expanded leaves present on the main axis of the seedling at the time of inoculation.

** B - Young expanding leaves present on lateral branches which were initiated after inoculation.

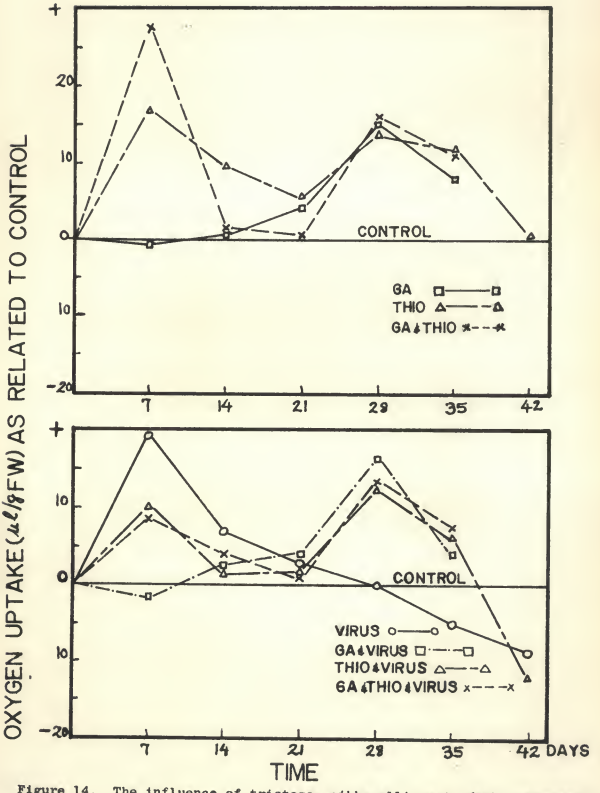

Figure 14. The influence of tristeza, gibberellic acid (GA), thiouracil (Thio), and interactions of these agents on the gaseous oxygen exchange pattern of 'Key' lime leaves.

Before studying the interactions between tristeza and the two chemicals, consider first the influence of GA and thiouracil on the plants. In Table 5 and Figure 14 it can be seen that both agents stimulated oxygen uptake. GA had very little influence on the consumption of oxygen until the twenty-eighth and thirty-fifth sampling dates, which amounted to an increase of 59 per cent and 22 per cent above the control. Thiouracil significantly increased oxygen uptake at all sampling dates after the time of inoculation, with the greatest stimulation occurring on the seventh and twenty-eighth days. A mixture of the two chemicals resulted in an M-shaped pattern with a stimulation of oxygen consumption occurring on the seventh, twenty-eighth and thirty-fifth sampling dates.

Next, consider the interaction between the virus and the chemicals on the pattern of oxygen consumption by the host tissue, again using the data in Table 5 and Figure 14. First, treatment of plants with GA and tristeza resulted in an oxygen uptake pattern very similar to that of GA alone. The stimulation of oxygen consumption by the virus in the earlier stages of infection was absent. Also, at the stage where the virus was inhibiting oxygen uptake, a combination of GA and tristeza was promoting oxygen consumption. Second, in the case of thiouracil in combination with the virus or in combination with GA and virus, the pattern of oxygen consumption was very similar to that of plant treated only with thiouracil. Thus, it was apparent that the influence of the virus on oxygen consumption was not stable and could be modified by chemical treatment.

Determinations of alcohol-soluble sugars: When 80 per cent ethanol extracts of leaf samples were examined periodically for hydrolyzable and reducing sugars throughout the duration of the tests, the major changes were in the sugar fraction that was hydrolyzable by invertase. This can be seen by comparing the data in Table 6 and Figure 15 with that in Table 7 and Figure 16. In the case of the hydrolyzable sugars, presumably sucrose, tristeza seems to have little effect on this fraction. There was a consistently slight decrease in hydrolyzable sugars at every sampling date after the day of inoculation, but statistically this was not significant. With GA and thiouracil, there was a significant increase in hydrolyzable sugars of the alcohol extract on a per cent fresh-weight basis, and a combination of the two chemicals had an even greater influence on this sugar fraction. Tristeza-infected plants treated with these chemicals also had greater quantities of alcohol-soluble hydrolyzable sugars on a fresh-weight basis than the controls (Fig. 15). When the non-infected plants treated with the chemical are compared to virus-infected similarly treated, the increase in hydrolyzable sugars of the latter was significantly less. The shape of curves of the chemical treatments on non-infected and virus-infected plants were very similar.

The changes in reducing sugars as a result of chemical treatment was evident, but they were of a lesser magnitude than changes in hydrolyzable sugars of the alcohol extract. In Table 7 and Figure 16 it can be seen that GA and thiouracil had a greater influence on non-infected than on virus-infected plants. Thus, it would seem that the influence of the various agents on reducing sugars was in the same direction as on alcohol-soluble hydrolyzable sugars, but of a lesser degree.

TABLE 6

THE INFLUENCE OF TRISTEZA, GIBBERELLIC ACID, AND THIOURACIL, ALONE AND IN VARIOUS COMBINATIONS, ON CHANGES IN HYDROLYZABLE SUGARS OF AN 80 PER CENT ETHANOL EXTRACT OF 'KEY' LIME LEAVES

Days after initiation of treatments	Source of sample	Tissue content of invertase hydrolyzable sugars (per cent of fresh weight)							
		Without Virus				With Virus			
		Control	GA	Thio	GA plus Thio	Control	GA	Thio	GA plus Thio
0	A*	1.72	1.72	1.72	1.72	1.72	1.72	1.72	1.72
7	A	1.95	2.40	2.47	2.71	1.84	2.23	2.31	2.01
14	A	2.06	2.66	3.28	3.82	1.95	2.17	2.61	2.42
21	A	2.13	2.74	2.50	3.81	2.06	2.65	2.40	2.89
28	A	2.14	2.73	2.28	3.79	2.04	2.47	2.30	3.17
35	B**	1.92	1.81	2.57	2.47	1.87	2.16	2.22	2.43
42	B	2.06	--	2.55	--	1.98	--	2.01	--

* A - Young fully expanded leaves present on the main axis of the seedling at the time of inoculation.

** B - Young expanding leaves present on lateral branches which were initiated after inoculation.

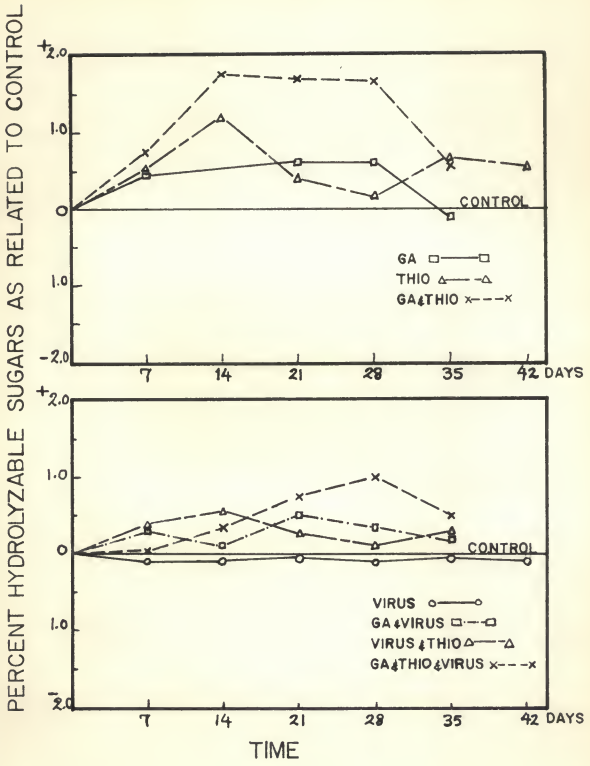

Figure 15. The influence of tristeza, gibberellic acid (GA), thiouracil (Thio), and interactions of these agents on hydrolyzable sugars of 'Key' lime leaves (hydrolyzable sugars of an 80 per cent ethanol extract).

TABLE 7

THE INFLUENCE OF TRISTEZA, GIBBERELLIC ACID, AND THIOURACIL, ALONE AND IN VARIOUS COMBINATIONS, ON CHANGES IN REDUCING SUGARS OF 'KEY' LIME LEAVES

Days after initiation of treatments	Source of Sample	Tissue content of reducing sugars (per cent of fresh weight)							
		Without Virus				With Virus			
		Control	GA	Thio	GA plus Thio	Control	GA	Thio	GA plus Thio
0	A*	1.11	1.11	1.11	1.11	1.11	1.11	1.11	1.11
7	A	1.09	1.17	1.29	1.31	1.07	1.15	1.24	1.04
14	A	1.12	1.32	1.55	1.58	1.18	1.15	1.34	1.24
21	A	1.15	1.31	1.53	1.40	1.02	1.19	1.13	1.23
28	A	1.21	1.38	1.62	1.42	1.05	1.38	1.23	1.30
35	B**	1.10	1.24	1.32	1.33	0.96	1.24	1.12	1.16
42	B	1.28	--	1.35	--	1.09	--	1.10	--

* A - Young fully expanded leaves present on the main axis of the seedling at the time of inoculation.

** B - Young expanding leaves present on lateral branches which were initiated after inoculation.

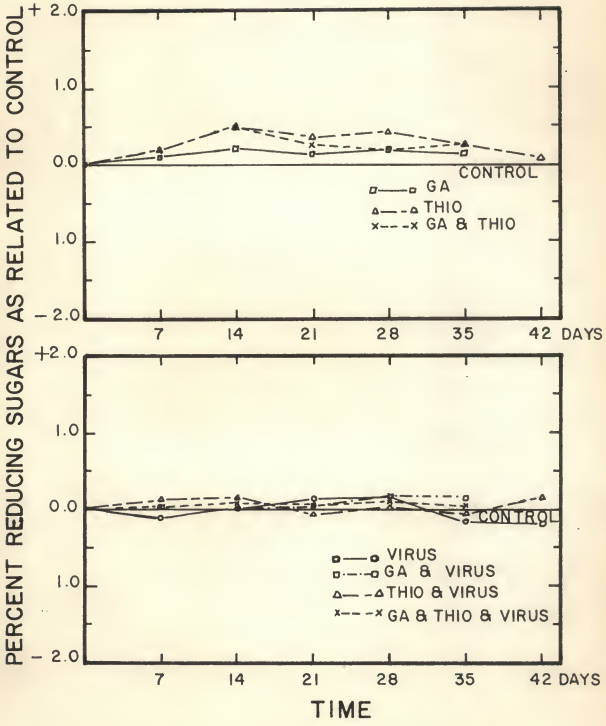

Figure 16. Effect of tristeza, gibberellic acid (GA), thiouracil, and interactions of these agents on reducing sugars of 'Key' lime leaves.

For convenience, the data of Tables 6 and 7 have been added together and included in Table 8 as total sugars of the extract. Since sugars hydrolyzable by invertase were the components influenced most, major differences noted in total sugars between treatments will be largely a reflection of changes in the fraction.

Determination of potassium: 'Key' lime plants, subjected to various treatments of tristeza, GA, thiouracil or of different combinations of these agents, seemed to contain less potassium than the control. This can be seen by a study of the data in Table 9. All the values are lower than the control, but only the plants treated with GA-plus-virus, thiouracil or thiouracil-plus-virus had potassium contents that were different enough from the control to be statistically significant at a high level.

Determination of calcium: There were no significant differences in the calcium content of tristeza infected and non-infected plants, as can be seen in Table 10. Also, infected plants treated with thiouracil had approximately the same amount of leaf calcium as the control. However, treatments of gibberellic acid, thiouracil, gibberellic acid-plus-thiouracil, virus-plus-gibberellic acid, and virus-plus-gibberellic acid-plus-thiouracil did influence the calcium levels of 'Key' lime leaves. Both non-infected and infected plants treated with gibberellic acid had significantly smaller amounts of calcium than the control--51 and 42 per cent respectively. Virus-free plants treated with thiouracil had a significantly higher calcium content than the control. Virus-free plants similarly treated with thiouracil-plus-gibberellic acid had a calcium

TABLE 8

THE INFLUENCE OF TRISTEZA, GIBBERELLIC ACID, AND THIOURACIL, ALONE AND IN VARIOUS COMBINATIONS, ON CHANGES IN ALCOHOL-SOLUBLE SUGARS OF 'KEY' LIME LEAVES

Days after initiation of treatments	Source of Sample	Tissue content of alcohol-soluble sugars (per cent of fresh weight)							
		Without Virus				With Virus			
		Control	GA	Thio	GA plus Thio	Control	GA	Thio	GA plus Thio
0	A*	2.83	2.83	2.83	2.83	2.83	2.83	2.83	2.83
7	A	3.04	3.57	3.76	4.02	2.91	3.38	3.55	3.05
14	A	3.18	3.98	4.83	5.40	3.13	3.32	3.65	3.66
21	A	3.28	4.05	4.03	5.21	3.08	3.84	3.74	4.12
28	A	3.35	4.11	3.90	5.21	3.09	3.85	3.63	4.47
35	B**	3.02	3.05	3.89	3.79	2.83	3.04	3.42	3.59
42	B	3.34	--	3.90	--	3.07	--	3.32	--

* A - Young fully expanded leaves present on the main axis of the seedling at the time of inoculation.

** B - Young expanding leaves present on lateral branches which were initiated after inoculation.

TABLE 9

THE INFLUENCE OF TRISTEZA, GIBBERELLIC ACID, AND THIOURACIL, ALONE AND IN VARIOUS COMBINATIONS, ON CHANGES IN POTASSIUM OF 'KEY' LIME LEAVES ON THE THIRTY-FIFTH DAY AFTER INITIATION OF THE TESTS

Per cent potassium on a dry weight basis							
Without Virus				With Virus			
Control	GA	Thio	GA plus Thio	Control	GA	Thio	GA plus Thio
3.07	2.32	2.20	2.57	2.27	1.80	1.77	2.65

TABLE 10

THE INFLUENCE OF TRISTEZA, GIBBERELLIC ACID, AND THIOURACIL, ALONE AND IN VARIOUS COMBINATIONS, ON CHANGES IN CALCIUM OF 'KEY' LIME LEAVES ON THE THIRTY-FIFTH DAY AFTER INITIATION OF THE TESTS

Per cent calcium on a dry weight basis							
Without Virus				With Virus			
Control	GA	Thio	GA plus Thio	Control	GA	Thio	GA plus Thio
1.30	0.75	1.90	0.75	1.20	0.64	1.10	0.67

content significantly lower than the control. Since all treatments with gibberellic acid, whether alone or in combination with tristeza or thiouracil, or both tristeza and thiouracil, decreased the calcium content of 'Key' lime leaves, it would seem that GA had the strongest influence on modifying tissue calcium. Thus, in treatments with tristeza or thiouracil, or both, GA masks their action.

Determination of transmissibility of virus

Observations on the interaction between tristeza and GA and thiouracil on 'Key' lime seedlings indicated that symptomatic changes were much faster with the chemical treatments. To determine whether this influence of the chemical was mainly on the physiology of the host or on the viral particle and to insure that the plants designated tristeza-infected or non-infected were properly classified, virus-free 'Key' lime seedlings were grafted with leaf pieces from the previous treatments. From these tests it was apparent that plants grafted with leaf pieces from the virus-plus-GA-treated plants had an accelerated symptomatic pattern. The onset of visual symptoms, in this case, was approximately six days earlier than on plants inoculated with a leaf piece from only tristeza-infected material. At the time visible symptoms occurred on the latter, the 'Key' lime seedlings, inoculated from the virus-plus-GA source, were at an advanced stage of infection, characterized by tip necrosis, abscission of young embryonic leaves, and pronounced vein clearing. A comparison of the two treatments on the fortieth day after grafting can be made by referring to Figures 17 and 18. Even after sufficient time had elapsed for both treatments to be in a severe stage of

Figure 17. Illustration showing corky vein as the result of gibberellic acid and thiouracil treatment.

Figure 18. Photograph of the apical portion of a branch of 'Key' lime infected with tristeza by grafting onto the seedlings, a leaf piece from the virus-infected plants of the first series of tests.

infection, the plants treated with inoculum from virus-plus-GA-infected seedlings seemed to have been affected to a greater extent.

After three to four months, the leaves on the plants infected with the inoculum from the virus-plus-GA treatment had prominent corky veins, while those infected with inoculum from only the tristeza-infected plants had not formed the corky-vein symptom after six months (see Fig. 17).

Caution must be taken in interpreting these results, for plants grafted with leaf pieces obtained from virus-free 'Key' lime seedlings treated with GA were markedly different from the controls on these plants. The rate of growth by stem elongation was much greater. Also, the leaves were leathery and yellow, as compared to the control which had normal dark green leaves. A representative leaf from each treatment can be seen in Figure 20.

Thiouracil also accelerated symptomatic changes associated with tristeza. The 'Key' lime seedlings receiving an inoculum from the tristeza-plus-thiouracil treatment had visible symptoms that were evident approximately three days earlier than on the seedlings receiving the inoculum from only the tristeza treatment. Compare Figures 17 and 21 for symptoms on the two treatments after forty days; also Figure 19 for appearance of the leaves. From the rate of symptomatic changes, the inoculum from the tristeza-plus-thiouracil treatment seemed to cause a reaction intermediate between inoculum from the tristeza-plus-GA and the tristeza treatment.

When the inoculum was obtained from the tristeza-plus-GA-plus-thiouracil treatment, the symptomatic reaction was very similar to that

Figure 19. Photograph of the apical portion of the branch of 'Key' lime infected with tristeza by grafting onto the seedling, a leaf piece from the virus-plus-GA treatment of the first series of tests.

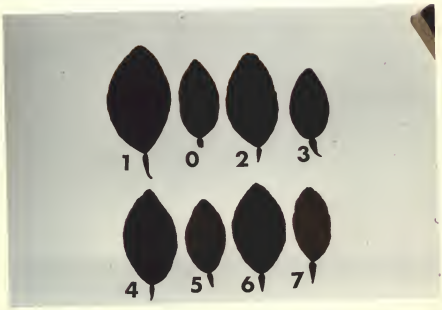

Figure 20. Photograph of representative leaves from 'Key' lime seedlings that were grafted with a leaf piece from one of the treatments in the first series of the tests.

Figure 21. Photograph of the apical portion of a branch of 'Key' lime infected with tristeza by grafting onto the seedlings a leaf piece from the virus-plus-thiouracil treatment of the first series of tests.

Figure 22. Photograph of the apical portion of the branch of 'Key' lime infected with tristeza by grafting onto the seedling a leaf piece from the virus-plus-GA-plus-thiouracil treatments of the first series of tests.

of plants infected with inoculum from the tristeza-plus-GA treatment. To obtain a comparison of the treatments refer to Figures 17, 18, 19, 20, 21 and 22.

To test again for the stability and transmissibility of the virus, inoculum from the various treatments of the first transfer was applied to virus-free 'Key' lime seedlings. In this second transfer test the observed rate of viral symptomatic development and the degree to which the plants were affected by the various inoculum were approximately the same as in the first transfer test. In the case of grafted seedlings with leaf pieces from only the chemical treatments, GA still had a noticeable effect, but thiouracil was without any influence.

From the grafting test it was apparent that no difficulty was evident in transmitting the virus. Also, the chemicals had either a permanent influence on the viral particle per se or the residual influence of the chemical was transmissible along with the virus, and the action of the two agents resulted in an accelerated symptomatic response of the host.

Experiment II

Influence of different concentrations of gibberellic acid on tristeza virus in 'Key' lime

Since a possibility existed that the chemicals were modifying the action of virus on the host, a second experiment was conducted to test the influence of various concentrations of GA on the virus-host response by treating a viral-infected leaf and then grafting it onto a virus-free 'Key' lime seedling. From this test, it was also apparent that GA was modifying the influence of the virus on the host.

The appearance of vein clearing, tip necrosis, and abscission of leaves were observed six to eight days earlier in the plants grafted with infected leaf pieces, soaked in GA (10^{-3} and 10^{-4} M), as compared to the plants grafted with infected leaf pieces soaked in distilled water. However, plants grafted with the viral-infected leaves, soaked in distilled water, had not shown any visible symptom of virus after 26 days. The appearance of these plants was very similar to those grafted with the virus-free leaf pieces soaked in 10^{-5} M GA alone.

Data in Table 11 and Figure 23 indicate that virus also inhibited the length of lateral branches significantly, as compared to control. The length of lateral branches was stimulated significantly by concentrations of 10^{-3} and 10^{-4} M of GA. There was no significant difference in the length of lateral branches of the plants which were grafted with leaf pieces soaked in 10^{-5} M GA.

Gibberellic acid at concentrations of 10^{-3} M and 10^{-4} M with virus-infected leaves suppressed growth of lateral branches when compared to treatment by a comparable GA treatment without virus. The 10^{-5} M concentration of GA overcame the suppression of growth caused by tristeza alone. Also, there was no significant difference between the plants grafted with the infected leaf pieces soaked in 10^{-5} M GA and virus-free leaf pieces soaked in 10^{-5} M GA.

Experiment III

The last series of tests concerned the influence of tristeza on changes in oxygen uptake, alcohol-soluble amino acids (free amino acids), and acid-soluble nucleotides.

TABLE 11

THE INFLUENCE OF DIFFERENT CONCENTRATIONS OF GIBBERELLIC ACID, ALONE AND IN COMBINATION WITH TRISTEZA, ON LENGTH OF LATERAL BRANCHES AS MEASURED FOUR MONTHS AFTER INFECTION

Treatment	Length of lateral branches (Cm)
10^{-3} M GA	22.8
10^{-4} M GA	17.9
10^{-5} M GA	14.1
0 M GA	14.0
Virus	3.8
Virus plus 10^{-3} M GA	7.8
Virus plus 10^{-4} M GA	7.2
Virus plus 10^{-5} M GA	13.0

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Square	F
Treatment	7	530.51	75.78	47.96**
Block	1	0.27	0.27	NS
Interaction	7	11.06	1.58	
Total	15	541.84		

Figure 23. Comparison of 'Key' lime seedlings infected with tristeza by grafting onto the plants a leaf piece infected with tristeza and treated by soaking in distilled water, 10^{-3} M (2), 10^{-4} M (3), and 10^{-5} M (4) GA.

Total oxygen uptake

As in Experiment I, total oxygen uptake was increased at the early stage of infection. As shown in Table 12, maximum respiration was on the seventh day after infection and after that it declined. On the twenty-eighth day after infection, infected leaves respired significantly less than the viral-free material.

Alcohol-soluble amino acids

Infected and non-infected tissues of 'Key' lime were analyzed for free amino acids on the seventh and twenty-eighth day after infection. Free amino acids in the leaves of infected and non-infected 'Key' lime seedlings on the seventh and twenty-eighth day after the initiation of the tests are listed in Tables 13 and 14. Glycine, serine, cysteine, lysine, histidine, arginine, alanine, citrulline, tryptophan, and proline were found in healthy leaves.

Chromatograms of extracts made of tissue on the seventh day of the test revealed that lysine was absent from viral-infected tissue, but was easily detectable in healthy tissues (Fig. 24). Qualitative measurements, mainly based on intensity of color and area of individual spots, indicated that there was a large decrease in cysteine, glycine, serine, alanine, tryptophan, and proline and a smaller decrease in citrulline and arginine.

On the twenty-eighth day of the test the number and kinds of alcohol-soluble free amino acids of viral-infected tissue was the same as the non-infected tissue, as can be seen in Table 14. However, the amounts present were still less in the infected tissue. This is also

TABLE 12

THE INFLUENCE OF TRISTEZA ON THE OXYGEN UPTAKE OF 'KEY' LIME LEAF DISCS ON THE TWENTY-EIGHTH DAY OF THE TEST

Days after initiation of treatments	Oxygen uptake (µl/g F.W.) of 'Key' lime leaf discs	
	Non-infected	Viral infected
0	26.89	26.89
4	30.76	38.11
7	34.42	45.46
14	36.16	39.38
28	35.66	27.92

TABLE 13

THE INFLUENCE OF TRISTEZA ON ALCOHOL-SOLUBLE AMINO ACIDS OF 'KEY' LIME TISSUE AFTER SEVEN DAYS OF INFECTION

Alcohol soluble amino acid	Rf values					
	Standard		Non-infected		Infected	
	Solvent		Solvent		Solvent	
	I	II	I	II	I	II
Cysteine	0.06	0.19	0.08	0.18	0.06	0.17
Serine	0.14	0.33	0.15	0.31	0.17	0.31
Glycine	0.17	0.37	0.16	0.37	0.17	0.36
Lysine	0.07	0.32	0.08	0.33	-	-
Histidine	0.08	0.45	0.12	0.43	0.07	0.42
Arginine	0.12	0.60	0.12	0.61	0.10	0.58
Alanine	0.24	0.56	0.26	0.59	0.20	0.58
Citrulline	0.12	0.68	0.13	0.74	0.10	0.70
Tryptophan	0.43	0.77	0.46	0.75	0.40	0.75
Proline	0.28	0.85	0.30	0.88	0.26	0.86

Solvent I - n-butanol:acetic acid:water (4:1:5); Solvent II - Phenol:water (5:1)
Filter paper- Whatman No. 1.
Source of Standards: F. H. Sargent & Co.

Figure 24. Photograph of a two-dimensional chromatogram of the alcohol-soluble amino acids of non-infected 'Key' lime seedlings on the seventh day after initiation of the tests.

TABLE 14

THE INFLUENCE OF TRISTEZA ON ALCOHOL-SOLUBLE AMINO ACIDS OF 'KEY' LIME TISSUE AFTER 28 DAYS OF INFECTION

Alcohol soluble amino acid	Rf values					
	Standard		Non-infected		Infected	
	Solvent		Solvent		Solvent	
	I	II	I	II	I	II
Cysteine	0.23	0.23	0.21	0.24	0.23	0.22
Serine	0.28	0.38	0.24	0.40	0.26	0.39
Glycine	0.24	0.42	0.26	0.42	0.24	0.41
Lysine	0.17	0.40	0.19	0.42	0.18	0.40
Histidine	0.19	0.48	0.19	0.50	0.19	0.50
Arginine	0.24	0.61	0.22	0.63	0.22	0.62
Alanine	0.38	0.67	0.39	0.65	0.37	0.66
Citrulline	0.24	0.66	0.24	0.65	0.24	0.65
Tryptophan	0.57	0.81	0.58	0.81	0.56	0.80
Proline	0.42	0.90	0.43	0.90	0.43	0.90

Solvent I - n-butanol:acetic acid:water (4:1:5); Solvent II-Phenol:water (5:1)
Filter paper - Whatman No. 4.
Source of Standards: F. H. Sargent & Co.

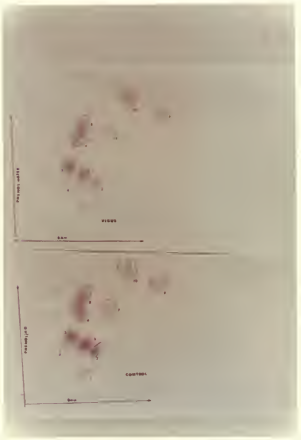

Figure 25. Photograph of a two-dimensional chromatogram of the alcohol-soluble amino acids of non-infected and infected 'Key' lime seedlings on the twenty-eighth day after initiation of the tests.

shown in Figure 25. Lysine was very faint in color on the chromatograms, which indicated that lysine was present in the infected tissue on the twenty-eighth day after infection, but in lesser extent than control. There were smaller qualitative changes in cysteine, glycine, serine, alanine, and tryptophane than in the controls, but no perceptible changes in citrulline, arginine, and proline. The qualitative comparison was made on the basis of intensity of the color and size of spot.

Determinations of acid-soluble nucleotides

Before analyzing plant samples for acid-soluble nucleotides, a mixture of known nucleotides was separated and analyzed by the same procedures that would later be applied to the extract. The pattern of separation of this mixture, afforded by the column, can be seen in Figure 26. All of the nucleotides added to the column were recovered. It was possible to determine from the recovery rates the dilution factor to be used later in calculation of the amount of a given nucleotide present in the plant extract. Spectroscopic analysis of these samples yielded the data presented in Table 15. It was possible to identify the components of the standard mixture without any difficulty, including AMP and CMP which are normally almost impossible to separate (24).

After the technique was standardized, infected and non-infected tissues of 'Key' lime were analyzed at 0, 7, 14 and 28 days after treatment. After the fractions were tentatively identified, the amount of nucleotides was calculated in each sample. Elution-chromatograms in Figures 27 to 33 show the nucleotides present in the infected and non-infected sample. Tables 15 to 22 indicate the possible nucleotides identified by spectroscopic analysis.

TABLE 15

SPECTROSCOPIC ANALYSIS OF FRACTIONS OF A KNOWN MIXTURE OF 5'-RIBONUCLEOTIDES SEPARATED ON A DOWEX 1 ION-EXCHANGE COLUMN

Absorbance Max/Min(mμ)		Ratio at pH 7		Possible Nucleotide
pH 2	pH 7	250/260	280/260	
280/245	275/250	0.84	0.98	CMP
258/235	260/240	0.83	0.92	DPN, TPN
280/245	271/250	0.86	0.94	CYTIDINE
258/230	256/230	0.79	0.14	AMP
258/230	253/225	1.15	0.66	GMP
280/242	270/250	0.83	0.97	CDP
265/235	261/230	0.70	0.39	UMP
262/235	272/240	0.75	0.36	URIDINE
258/232	258/230	0.78	0.14	ADP
258/230	252/228	1.15	0.66	GDP
280/242	271/250	0.86	1.37	CTP
265/235	261/230	0.74	0.41	UDP
257/230	260/228	0.82	0.17	ATP
256/228	252/225	1.20	0.64	GTP
265/232	265/230	0.76	0.38	UTP

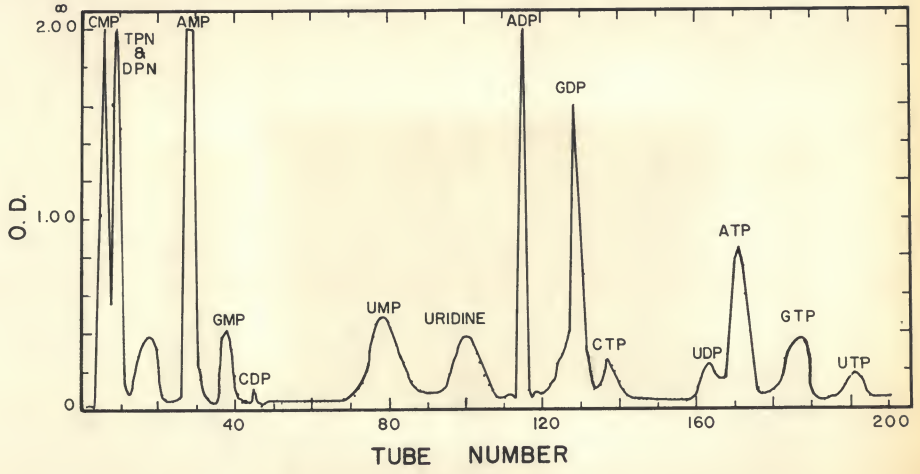

Figure 26. Elution chromatogram of 5'-Ribonucleotides at 260 um.

TABLE 16

TENTATIVE IDENTIFICATION OF ACID-SOLUBLE NUCLEOTIDES OF NON-INFECTED TISSUE (AT TIME OF INFECTION) OF 'KEY' LIME BY SPECTROSCOPIC ANALYSIS

Absorbance Max/Min(mu)		Ratio at pH 7		Possible
pH 2	pH 7	250/260	280/260	Nucleotide
280/250	275/250	0.85	0.96	CMP
260/232	256/230	0.80	0.12	AMP
255/230	250/225	1.20	0.70	GMP
280/250	278/255	0.84	0.95	CDP
265/240	260/240	0.78	0.40	UMP
255/235	258/248	1.30	0.65	GDP
280/245	278/250	0.90	1.40	CTP
268/235	265/238	0.75	0.36	UTP

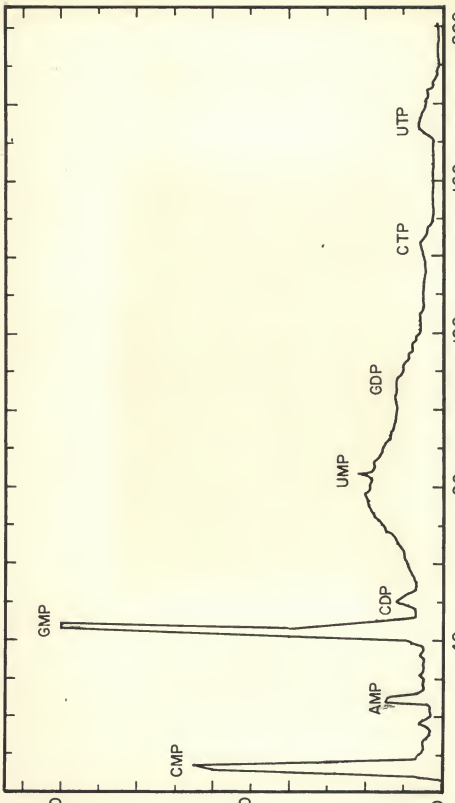

Figure 27. Elution chromatogram of soluble nucleotides from seven grams (FW) from non-infected tissue of 'Key' lime in the day of infection.

TABLE 17

TENTATIVE IDENTIFICATION OF ACID-SOLUBLE NUCLEOTIDES OF NON-INFECTED TISSUE (SEVEN DAYS AFTER TREATMENT) OF 'KEY' LIME BY SPECTROSCOPIC ANALYSIS

Absorbance Max/Min(mμ)		Ratio at pH 7		Possible Nucleotide
pH 2	pH 7	250/260	280/260	
280/255	278/250	0.84	0.97	CMP
260/235	258/232	0.79	0.13	AMP
250/225	250/230	1.19	0.81	GMP
280/250	278/255	0.85	0.96	CDP
265/240	262/245	0.76	0.41	UMP
255/235	256/230	1.25	0.63	GDP
278/250	280/250	0.95	1.38	CTP
270/235	265/235	0.81	0.36	UTP

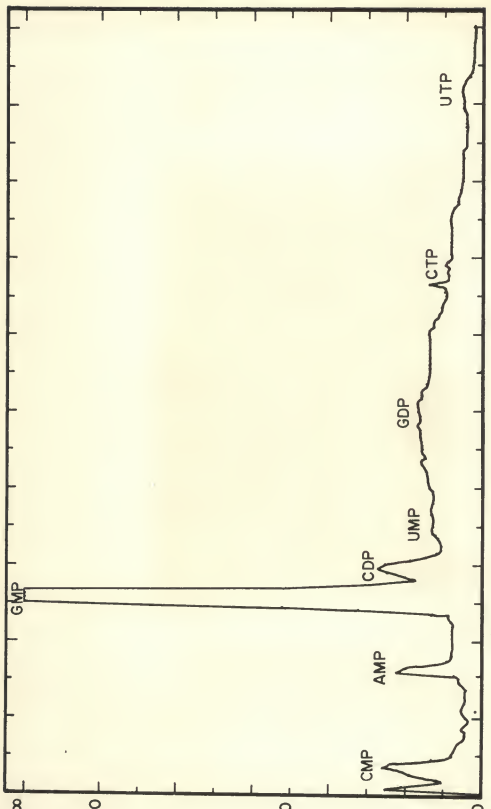

Figure 28. Elution chromatogram of soluble nucleotides from seven grams (FW) from non-infected leaf-tissue of 'Key' lime on the seventh day after treatment.

TABLE 18

TENTATIVE IDENTIFICATION OF ACID-SOLUBLE NUCLEOTIDES OF VIRUS-INFECTED TISSUE (SEVEN DAYS AFTER INFECTION) OF 'KEY' LIME BY SPECTROSCOPIC ANALYSIS

Absorbance Max/Min(mu)		Ratio at pH 7		Possible
pH 2	pH 7	250/260	280/260	Nucleotide
278/250	280/250	0.84	0.97	CMP
260/245	258/240	0.77	0.99	AMP
250/225	250/230	1.10	0.70	GMP
280/255	278/250	1.00	1.10	CDP
272/245	265/248	0.68	0.42	UMP
260/235	258/240	0.90	0.16	ADP
255/230	250/230	1.20	0.71	GDP
280/250	280/255	0.89	1.43	CTP
260/240	260/240	0.87	0.16	ATP
256/230	252/225	1.20	0.61	GTP
270/245	272/245	0.82	0.41	UTP

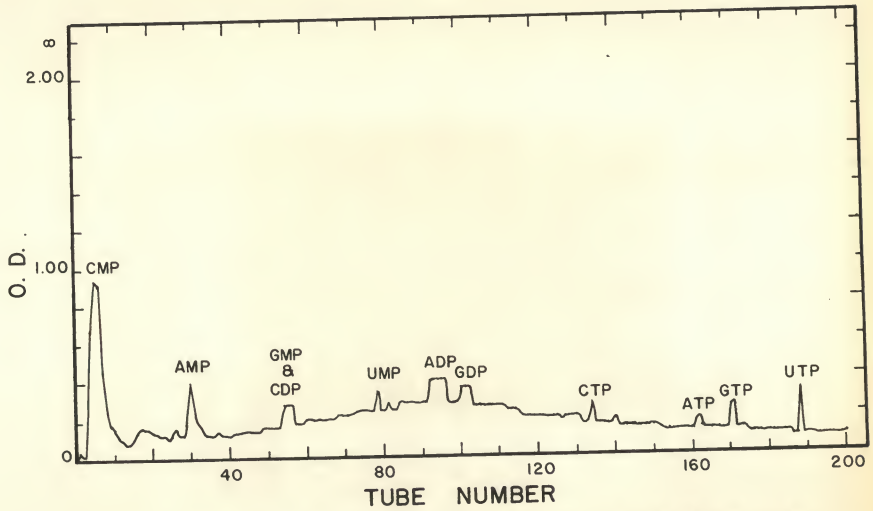

Figure 29. Elution chromatogram of soluble nucleotides from four grams (FW) from virus-infected leaf tissue of 'Key' lime on the seventh day after infection.

TABLE 19

TENTATIVE IDENTIFICATION OF ACID-SOLUBLE NUCLEOTIDES OF NON-INFECTED TISSUE (14 DAYS AFTER TREATMENT) OF 'KEY' LIME BY SPECTROSCOPIC ANALYSIS

Absorbance Max/Min(mμ)		Ratio at pH 7		Possible
pH 2	pH 7	250/260	280/260	Nucleotide
280/248	278/250	0.86	0.96	CMP
260/240	258/235	0.78	1.00	AMP
255/225	250/225	1.20	0.65	GMP
280/255	275/250	0.92	1.00	CDP
268/245	265/240	0.68	0.42	UMP
260/235	258/238	0.81	0.20	ADP
256/235	250/240	1.20	0.68	GDP
270/240	265/240	0.81	0.43	UDP
280/250	280.245	0.91	1.40	CTP
260.240	265/242	0.89	0.16	ATP
256/225	252/230	1.25	0.62	GTP
270/240	265/235	0.81	0.41	UTP

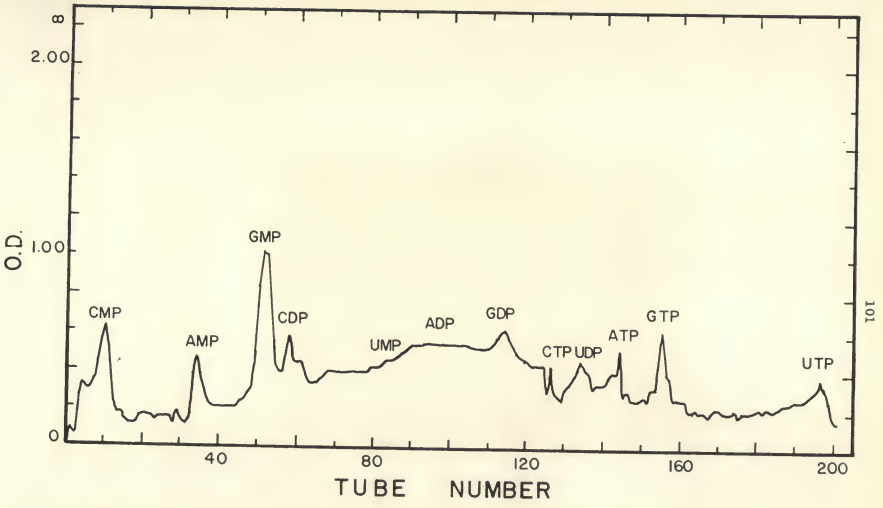

Figure 30. Elution chromatogram of soluble nucleotides from seven grams (FW) from non-infected leaf tissue of 'Key' lime on the fourteenth day after treatment.

TABLE 20

TENTATIVE IDENTIFICATION OF ACID-SOLUBLE NUCLEOTIDES OF VIRUS-INFECTED TISSUE (14 DAYS AFTER INFECTION) OF 'KEY' LIME BY SPECTROSCOPIC ANALYSIS

Absorbance Max/Min(mμ)		Ratio at pH 7		Possible Nucleotide
pH 2	pH 7	250/260	280/260	
280/248	280/250	0.83	0.98	CMP
258/250	260/245	0.78	0.97	AMP
250/225	250/225	1.00	0.69	GMP
280/250	278/250	1.10	1.00	CDP
270/250	272/248	0.71	0.40	UMP
256/240	258/235	0.87	0.14	ADP
250/230	255/225	1.18	0.72	GDP
278/250	275/245	0.87	1.32	CTP
260/240	258/235	0.85	0.18	ATP
255/230	258/230	1.18	0.67	GTP
270/245	268/245	0.80	0.47	UTP

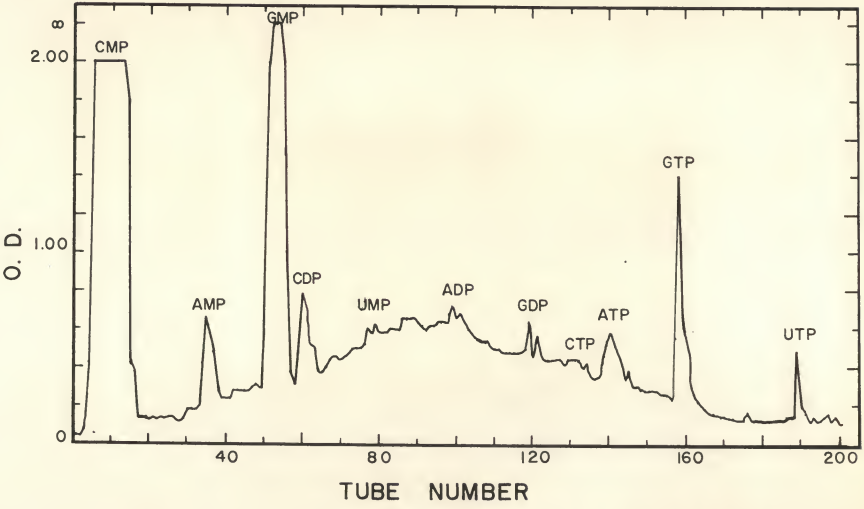

Figure 31. Elution chromatogram of soluble nucleotides from ten grams (FW) from virus-infected leaf tissue of 'Key' lime on the fourteenth day after infection.

TABLE 21

TENTATIVE IDENTIFICATION OF ACID-SOLUBLE NUCLEOTIDES OF NON-INFECTED TISSUE (28 DAYS AFTER TREATMENT) OF 'KEY' LIME BY SPECTROSCOPIC ANALYSIS

Absorbance Max/Min(mμ)		Ratio at pH 7		Possible Nucleotide
pH 2	pH 7	250/260	280/260	
278/250	280/250	0.85	0.94	CMP
260/240	258/240	0.77	1.10	AMP
255/230	250/225	1.10	0.67	GMP
280/255	275/250	0.90	1.10	CDP
270/245	265/240	0.67	0.40	UMP
260/235	260/240	0.81	0.18	ADP
256/235	252/240	1.21	0.70	GDP
270/245	265/245	0.80	0.42	UDP
280/250	280/255	0.83	1.37	CTP
260/245	260/250	0.85	0.18	ATP
256/225	250/225	1.21	0.63	GTP
270/245	265/240	0.80	0.42	UTP

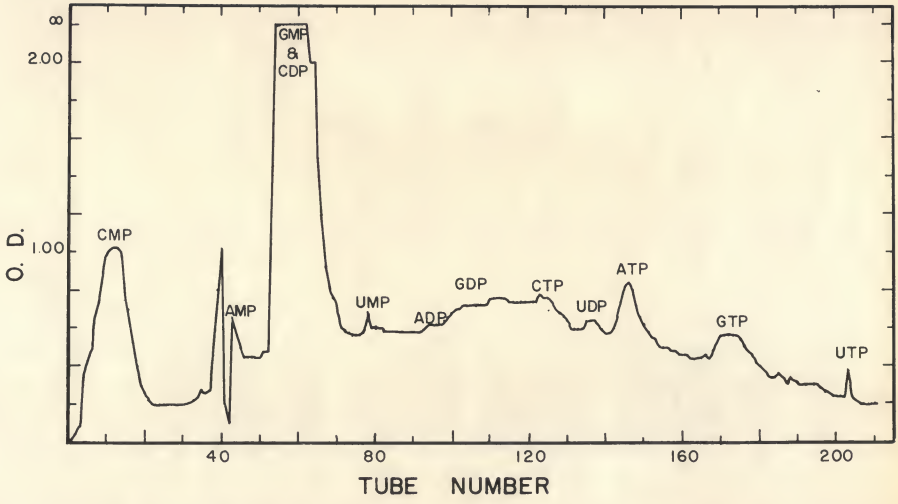

Figure 32. Elution chromatogram of soluble nucleotides from ten grams (FW) from non-infected leaf tissue of 'Key' lime on twenty-eighth day after treatment.

TABLE 22

TENTATIVE IDENTIFICATION OF ACID-SOLUBLE NUCLEOTIDES OF VIRUS-INFECTED TISSUE (28 DAYS AFTER INFECTION) OF 'KEY' LIME BY SPECTROSCOPIC ANALYSIS

Absorbance Max/Min(mμ)		Ratio at pH 7		Possible
pH 2	pH 7	250/260	280/260	Nucleotide
278/255	280/250	0.84	0.96	CMP
260/240	260/240	0.77	0.99	AMP
255/230	250/230	1.10	0.66	GMP
280/255	275/255	0.90	1.00	CDP
270/250	265/245	0.70	0.40	UMP
260/235	260/240	0.81	0.18	ADP
258/240	252/240	1.19	0.72	GDP
280/252	280/255	0.84	1.30	CTP
260/245	260/250	0.85	0.18	ATP
256/225	250/225	1.20	0.63	GTP
270/245	265/240	0.80	0.42	UTP

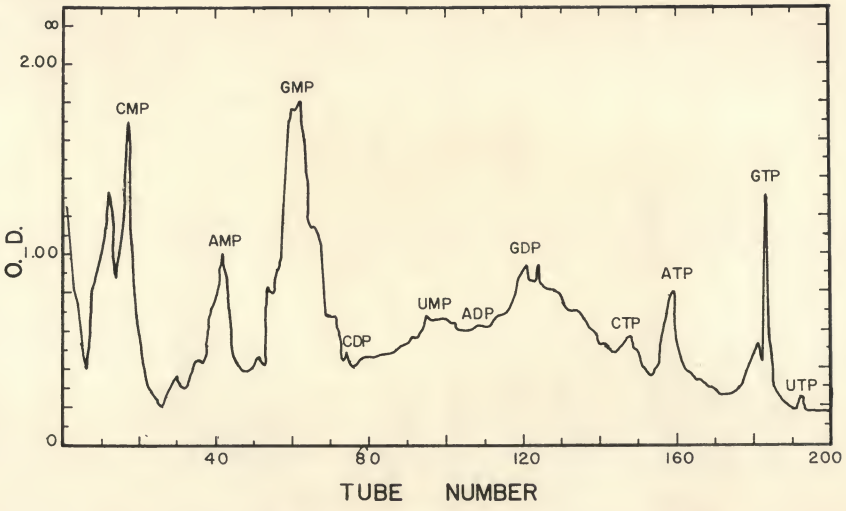

Figure 33. Elution chromatogram of soluble nucleotides from ten grams (FW) from virus-infected leaf tissue of 'Key' lime on twenty-eighth day after infection.

Table 23 shows the changes in nucleotides at 7, 14 and 28 days after infection. On the seventh day after infection there was considerable increase (39 per cent) in total adenosine mono-, di-, and tri-phosphates. The content of ATP, ADP, and AMP were higher than the control. Especially, the amount of ADP in infected plants increased considerably, as compared to content of ADP in non-infected plants. The total adenosine phosphates decreased on the fourteenth day after infection and by the twenty-eighth day a significant increase in the total adenosine phosphates did not exist. On the twenty-eighth day, the significant drop (30 per cent) was noted in the content of ADP of infected plants, as compared to control. On the twenty-eighth day, the amount of AMP in the infected plants was 157 per cent more than control, while the content of ATP was lower. In sum, after 28 days of infection, there was no significant difference between virus-infected and non-infected plants in the amount of adenosine phosphates measured in this experiment.

At 0, 7, 14 and 28 days after infection, the infected and non-infected tissues did not differ significantly in the amount of total cytosine mono-, di-, and, tri-phosphates. The amount of CTP did not differ significantly after total 28 days of infection, while there was a significant increase in the amount of CMP and decrease in CDP. On the seventh and fourteenth days after the infection, the amount of CMP was higher in the infected tissue than non-infected tissue. On the seventh, fourteenth, and twenty-eighth days, the content of CDP in infected tissue was always lower than non-infected tissue. On the

TABLE 23

INFLUENCE OF TRISTEZA ON ACID-SOLUBLE NUCLEOTIDES OF 'KEY' LIME TISSUE (ug/g FRESH WEIGHT)

Nucleotide	Without Virus					With Virus				
	Days after treatment					Days after infection				
	0	7	14	28	Total	0	7	14	28	Total
ATP	-	-	114	172	286	-	49	82	97	228
ADP	-	-	288	72	360	-	212	68	80	360
AMP	45	82	120	55	302	45	139	68	144	396
	45	82	522	299	948	45	400	218	321	985
CTP	40	39	149	62	290	40	81	53	115	289
CDP	58	190	113	62	423	58	58	96	35	247
CMP	160	129	198	238	725	160	341	365	162	1028
	258	358	460	362	1438	258	480	514	312	1564
GTP	-	-	196	155	351	-	77	112	64	253
GDP	89	136	221	162	608	89	166	46	95	396
GMP	39	821	366	1357	2583	39	104	335	500	978
	128	957	783	1674	3542	128	347	493	659	1627
UTP	47	49	135	22	253	47	70	24	17	158
UDP	-	-	73	55	128	-	-	-	-	-
UMP	69	66	71	69	275	69	85	68	34	256
	116	115	279	146	656	116	155	92	51	414

fourteenth day the amount of CTP was also decreased in virus-infected plants, while the amount of CMP was higher in the infected tissues. On the twenty-eighth day, the amount of CTP was increased in the infected tissue, but the amount of CDP and CMP was lower than non-infected tissue.

The content of total guanosine mono-, di-, and tri-phosphates was lower on the seventh, fourteenth, and twenty-eighth days after infection. The amount of GTP, GDP, and GMP of infected tissues were always lower than the non-infected tissue, except on the seventh day, the amount of GTP and GDP of the infected tissue was higher than non-infected tissue. The maximum decrease was in the amount of GMP on the seventh and twenty-eighth daysafter infection. The amount of GMP of infected tissue was lower on the fourteenth day, but this decrease was very little.

Uridine diphosphate was never detected in tristeza-infected plants, while a measurable amount of UDP was detected on fourteenth and twenty-eighth days after treatment in healthy plants.

On the seventh day after infection, the amounts of total uridine mono- and tri-phosphates of the infected tissue were higher than the amount of total UTP, UDP, or UMP of non-infected tissue. After seven days of infection, the amounts of UTP and UMP decreased in the infected plants, especially in UTP, on the fourteenth day after infection.

In short, 28 days after infection, the maximum decreases were in the content of the combined GTP, GDP, and GMP or individually, as compared to the control. Total uridine and adenosine phosphates also decreased, but the changes in adenosine phosphates were not significant,

TABLE 24

INFLUENCE OF TRISTEZA ON TOTAL ACID-SOLUBLE NUCLEOTIDES OF 'KEY' LIME TISSUE (ug/g FRESH WEIGHT)

Days after infection	Non-infected	Infected
0	647	647
7	1512	1382
14	2044	1317
28	2481	1343

while the decreases in uracil phosphates were only 37 per cent. Cytosine phosphates were higher in virus-infected plants, but the increases were only about 15 per cent.

On the seventh day after infection, ATP, ADP, AMP, UTD, UMP, GTP, GDP, CTP, or CMP were higher, as compared to the control. Only CDP and GMP were lower in content in infected plants. On the fourteenth day, all of the nucleotides (Table 20) decreased, except CMP. The amount of CMP was significantly higher than control. On the twenty-eighth day, the only significant change was in AMP and CTP, while all of the nucleotides, except ADP were lower in content in virus-infected plants as compared to control.

Data in Table 23 indicate that pyrimidine phosphates (phosphates derivatives containing cytosine and uracil) were always lower, except on the seventh day after infection. There was an increase of 35 per cent in the amount of pyrimidine phosphates of infected tissues as compared to the content of pyrimidine phosphates of non-infected tissue. The significant and maximum decrease was in purine phosphates on every day of sampling.

The data in Table 24 indicate that as the infection advanced, there was an 8, 36, and 48 per cent decrease on the seventh, fourteenth, and twenty-eighth days, respectively, of total acid-soluble nucleotides in tristeza-infected 'Key' lime tissue, as compared to non-infected tissue.

DISCUSSION

Seedlings of 'Key' lime, infected with tristeza under controlled conditions, exhibited similar patterns of vein clearing, growth depression, leaf abscission and tip necrosis to those reported by Grant (59). Visible symptoms of viral infection became evident on the plants between the twenty-eighth and thirty-fifth days after inoculation. In general, there was a shorter time-lapse in these tests from inoculation to the appearance of noticeable symptoms than in most previous reports (58, 59, 62). However, this difference may have been due to the age of the plants or to environmental conditions, because it was possible to duplicate this shorter period of time to visible symptoms from test to test.

Tristeza had a marked influence on the oxygen uptake of 'Key' lime leaves. During the early stages of infection, from inoculation to the seventh day after inoculation, total oxygen uptake was much greater by the viral-infected tissue than by the non-infected tissue. Thereafter, oxygen uptake of the infected tissues steadily declined, until on the thirty-fifth day after infection it was significantly less than that of virus-free material. These observations were in agreement with the suggestion made by Millerd and Scott (91) that, in general, the respiration rate of diseased plants increases only during the early stages of infection, and thereafter the rate of oxygen uptake is less than that of non-diseased tissues. They came to this conclusion after

reviewing the reports on oxygen uptake of virus-infected and non-infected plants by numerous workers (100, 101, 102, 103, 128, 140).

On the other hand, these tests point to the fact that statements concerning the oxygen uptake of virus-infected tissues should be made with a great deal of caution, a suggestion made by several investigators previously (11, 12, 80). As was shown (Table 5 and Fig. 13), the treating of virus-infected plants with gibberellic acid completely reversed the pattern of oxygen uptake without hindering the multiplication of tristeza in 'Key' lime. In fact, symptomatic changes normally associated with tristeza infection of 'Key' lime were hastened and enhanced.

From the period of inoculation until visible symptoms of infection appeared on the plant, changes in total phosphorus followed a pattern very similar to that of oxygen uptake. Holden and Tracey (68) found a similar pattern of changes in total phosphorus with another type of systemic virus. It may be significant that mosaic-type viruses do not seem to have this influence on the phosphorus content of infected tissues. Vayonis (130) studied the changes in total phosphorus of tobacco leaves as influenced by TMV. He found that the total phosphorus content of diseased and non-diseased tissues were about the same until six days after inoculation. After that, it decreased markedly. In this same report, it was shown that the content of phosphorus in the tissue depended upon the time of sampling, stage of infection, age of the tissue, and on whether determinations were based on fresh weight or dry weight of the tissue. Thus, any suggestion that changes in tissue phosphorus were different for systemic and mosaic type of viruses is tentative.

Tristeza influenced the total nitrogen content of 'Key' lime tissue in a manner similar to that reported for other systemic viruses (99, 127). That is, tristeza-infected plants had less total nitrogen than non-infected plants (Table 3 and Fig. 11).

The carbohydrate content in the tissues of 'Key' lime was influenced very little following infection with tristeza, under the conditions of these tests (Tables 6, 7 and 8, and Figs. 15 and 16). This would seem to indicate that tristeza is slightly different from other systemic viruses, but as shown with other measurements of components in the system of the host, the relation between virus-infected and non-infected tissues is strongly dependent on the stage of infection, age and type of tissue, and environmental conditions.

When a balance of carbohydrates and nitrogen was considered, it was found to be in the same direction as that of the systemic type of viruses and opposite to that of the mosaic type of viruses. Dunlap (42) considers this a definitive characteristic of the type of virus.

The interactions between tristeza and GA and thiouracil on seedlings of 'Key' lime were complex. Explanations for all of the observed facts cannot be given because a complete understanding of the mechanisms of action of either of the agents alone is not known. However, several observations would seem worthy of mentioning. First, the suppression of growth of 'Key' lime seedlings by tristeza was not a result of limited substrates, at least during the stage of infection from inoculation to the appearance of visible symptoms. This can be surmised from a comparison of the rate of growth of virus-infected and non-infected

seedlings treated with GA. It would seem possible, as suggested by Chessin (26), that the suppression of growth of the host could be a result of an upset in the hormonal system of the plant. In this connection, Kutsky and Rawlins (77), Limasset et al. (79), and Grieve (64) have obtained therapeutic uses of auxin against tobacco mosaic viruses and the X and Y types of potato viruses, respectively.

One of the original reasons for studying the interaction of tristeza and GA on 'Key' lime was to determine whether or not GA could influence the degree of infectivity of the host by the virus. In the first series of tests GA, at an application rate calculated to produce a rapid rate of growth, hastened the symptomatic changes associated with tristeza and also intensified the severity of certain phenomena, e.g., abscission of young embryonic leaves, tip necrosis, and corky veins of the leaves. This was not a temporary relation between the host and tristeza, because it was transmissible through two graftages. Although this would seem to indicate that a more severe strain of virus had been induced by the conditions of these tests, some reservations must be attached to this idea for tissues from non-infected seedlings treated with GA, grafted onto normal seedlings also produced measurable stimulations of growth. It was observed that the influence of GA diminished with each transfer by grafting, while the symptomatic changes associated with the virus were more stable. A fact which would support the suggestion that the viral particles might possibly be affected per se, but this cannot be proven conclusively until tristeza particles have been isolated. At any rate, it would seem

reasonable to assume that GA does not adversely affect viral synthesis or multiplication.

There was a previous report by Maramorosch (83) that the stunting effect of certain viruses could be reversed by GA. He was able to counteract with GA the suppression of growth of plants infected with corn-stunt, aster-yellows, and wound-tumor viruses. However, the plants retained other characteristic symptoms of infection that are normally associated with the respective virus which agrees with the findings on the interaction between GA and tristeza on 'Key' lime.

To further support the idea that the phytohormonal status of a plant tissue could greatly influence the relation of a virus to the host, a critical examination of the results of the second test on the interaction of GA and tristeza on 'Key' lime will be discussed. When samples of viral-infected and non-infected 'Key' lime leaves were soaked in different concentrations of GA (10^{-3}, 10^{-4}, 10^{-5} M) and leaf pieces from each treatment were grafted onto virus-free 'Key' lime seedlings, it was evident that GA under the conditions of these tests was greatly modifying the influence of the virus on the host (Fig. 16). Plants grafted with a leaf piece from non-infected leaves soaked in either 10^{-3} or 10^{-4} M concentrations of GA stimulated elongation of the branches in a manner expected from previous work. Plants grafted with a leaf piece from the virus-infected leaves soaked in 10^{-3} or 10^{-4} M concentration of GA had a growth rate that resembled the plants grafted with non-infected leaf tissue treated with GA. Other symptomatic changes associated with tristeza were both hastened and intensified by the GA treatment. In contrast to this, plants grafted with

a piece of tissue from a virus-infected leaf soaked in 10^{-5} M concentration of GA was not visibly different after three months from plants receiving the non-infected tissue similarly treated. There has not been a sufficient lapse in time to determine whether or not the 'Key' lime plants are free from the virus. Thus, it would seem reasonable to assume that GA can modify the influence of tristeza on 'Key' lime. At high physiological concentrations it increases the severity of the virus reactions on the host; and at low concentrations, possibly physiological levels, it decreases the effect of the virus on the host.

Although the influence of GA on citrus was not a primary concern of this investigation, observations were made as a basis for determining the nature of the interactions between the chemical and tristeza. Some of these observations seem pertinent and worthy of mentioning briefly. The growth response of the seedlings to GA was typical of those observed on many species by numerous workers as reviewed by Stowe and Yamaki (123), Morgan and Mees (93), and Sachs (116). Also, 'Key' lime plants treated with GA had marked changes in oxygen uptake, total phosphorus, carbohydrates and total nitrogen. These changes were similar to those of other plants (37, 45, 74, 104, 105, 137). Furthermore, it was noted that the influence of GA on 'Key' lime could be transferred by grafting. The amount of residual GA in or on the small leaf piece used in the grafting could not have elicited the observed changes in growth. This would seem to indicate that GA had an influence on the treated tissue which subsequently was transmissible by graftage. The nature of this growth stimulation may be similar to that of pea sections as reported by Galston (53).

Investigations concerning the mechanism of action of GA on plant metabolism may afford some clues as to the nature of the interaction between tristeza and GA on the host. If auxins are involved, as indicated by the reports on the therapeutic uses of these materials on certain plants (26), it could be possible that GA affects the host-virus relation through auxins. High concentrations of GA caused tip necrosis which in due time would result in a decreased level of auxin in the tissue. As shown by several investigators (2, 53, 119), low tissue content of auxin seems to favor synthesis of precursors for lignification. Under these conditions, corky veination, such as observed, may be a symptom associated with the virus.

Low concentrations of GA could have a synergistic effect with auxin of the type reported by Brian and Hemming (18). This enhanced auxin action could have a therapeutic value for the host against the virus. Part of the therapeutic influence of auxin could be via nucleic acid metabolism, since it has been shown that auxin enhanced the production of nucleic acids (119).

Thiouracil, an analogue of uracil, has been reported to be an effective inhibitor of the biosynthesis of TMV (31). It was partially from the work with thiouracil that Bawden and Kassanis (9) and Mercer et al. (89) suggested that a uracil-demanding system of plants is involved in nucleic acid and protein synthesis. The inhibition of the biosynthesis of TMV by thiouracil was effective only when TMV-inoculated leaves were kept in light in a sucrose-phosphate buffer (11). In other reports, Porter and Weinstein (111) and Kurtzman et al.(76) observed that thiouracil-treated plants were stunted, dark

green in color and an analysis of the leaf tissue revealed lesser amounts of nitrogen, phosphorus, and carbohydrates than that of the control. Also from the work of Porter and Weinstein (111), it was shown that thiouracil was an effective inhibitor of TMV only at these relatively high concentrations, which also adversely influence the metabolism of the host.

Under the conditions of the present tests, thiouracil did not seem to influence the rate of growth of non-infected 'Key' lime seedlings. However, plants treated with the compound were found to contain greater quantities of nitrogen, phosphorus, and carbohydrates and exhibit a greater rate of oxygen uptake. These findings are not in agreement with those outlined above and it would seem possible that part of the discrepancy was a result of a difference in concentration of thiouracil used. In the case of 'Key' lime tissue treated with only 3 ppm of thiouracil weekly, the tissue content of uracil was probably sufficient to supply the uracil-demanding system for nucleic acid and protein synthesis that was associated with viral production. However, thiouracil was probably interfering with certain uracil-demanding systems in the cell, since plants treated with both tristeza and thiouracil showed visible symptoms of stress much sooner than plants only treated with tristeza.

When the interaction of GA, thiouracil, and tristeza were compared to other treatments, it was observed that this treatment was very similar to that of only GA and tristeza. There was no evidence for either an additive effect of the two chemicals on the response of the host to tristeza infection or a lessening of the influence of either of the chemicals by the other.

From an analysis of the free amino acids in virus-infected and non-infected leaves of 'Key' lime on the seventh and twenty-eighth days after inoculation, it was evident that changes in these constituents were taking place. Ten amino acids were consistently detectable in alcohol extracts of non-infected tissues, namely, cysteine, serine, glycine, lysine, histidine, arginine, alanine, citrulline, tryptophan, and proline. On the seventh day after inoculation, lysine was not evident in the extract from infected plants; and glycine, serine, alanine, and tryptophan were present in much lower quantities than in the control. On the twenty-eighth day after inoculation, all ten detectable amino acids were present in much lower quantities than in the control. Of these, the maximum reductions were in glycine, serine, alanine, tryptophan, and lysine.

These observations on the effect of systemic viruses on free amino acids of a host can be compared to those of Commoner and Nehari (32), who reported transitory deficiencies in amino acids, especially serine; Andreae and Thompson (2), who reported decreases in tryptophan; Perdrizet (107), who reported decreases in serine and proline; and Filippo and Grilli (52), who reported decreases in glycine, lysine, and histidine. In the case of mosaic viruses, investigations by Bailova-Yankulova (6), Magdoff et al. (82), and Porter (110) indicate that the content of free amino acids increases especially the amides.

The decrease in glycine and serine could possibly be a result of their role in the make-up of the carbon and nitrogen structure of purine and pyrimidine moieties of nucleotides. It would seem possible

during the early stages of infection there is a rapid synthesis of purine and pyrimidine rings which could draw heavily on glycine and serine.

Proline has been reported by Stewart (122) as an important storage source of nitrogen in citrus. Thus, this pool of nitrogen might play a role as a source of nitrogen in the synthesis of viral particles.

Tryptophan decreased as a result of tristeza infection. This could have an influence on auxin production in the tissue, which in turn could influence the interaction between the virus and the host. It is known that tryptophan is a precursor of β-indoleacetic acid.

Absence of lysine on the seventh day after infection might be understood by assuming a possible acceleration of the following metabolic pathway: α-ketoglutarate, a precursor of both lysine and glutamic acid, is diverted to the synthesis of glutamic or glutamine. Then, this compound is directly involved in the synthesis of purines and pyrimidines. During the early stages of infection there is more than likely a rapid synthesis of viral particles, which depends on nucleotides (5).

As shown by the data in this report (Tables 23 and 24, and Figs. 26-33), there are marked differences between the soluble nucleotides of virus-infected and non-infected plants. This was in line with what could be expected, since some nucleotides are precursors for the RNA portion of the viral particle and other nucleotides are intimately involved in cellular metabolism. A significant finding

could be the observation that nucleotide changes of virus-infected tissues showed some similarities to those reported for tissues subjected to conditions of water stress (134), X-irradiation of corn seeds (25), and potassium deficiency (94). That is, the shift in the ratio of AMP plus CMP to GMP plus UMP. On the other hand, there was a marked difference between the observations of this investigation and those on changes in nucleotides of tissues under stress.

Cherry et al. (25) and West (134) found an increase in total nucleotide content of tissues during the early stage of treatment. The total nucleotide content of tristeza infected 'Key' lime tissue was less than that of the control on the first sampling date and continued to decrease as infection progressed. This decrease in total nucleotide could possibly be due to utilization of nucleotides in the synthesis of viral particles. In the experiment of West (134) and Cherry et al. (25) there was no synthesis of a foreign particle to utilize the nucleotides.

The difference in the total content of adenosine phosphates and cytosine phosphates between virus-infected and non-infected plants was not significant at the end of forty-two days. This was similar to the results of West (134) for these two compounds of corn plants grown under water stress. The content of guanosine and uridine phosphates was lower in virus-infected than in non-infected plants. Here again, there was a difference between tissue infected with a virus and tissue subjected to stress. West (134) has shown just the opposite to be true with corn plants grown under water stress. When it is considered that viral synthesis would use guanosine and uridine phosphates, then this difference may be understandable.

The sum of adenine and uracil phosphates was higher than the sum of cytosine and guanine phosphates in virus-infected tissues. This indicates that the bases cytosine and guanosine are depleted during the period of infection to a much larger extent than adenosine and uracil.

Several conditions for stimulation of respiration have been proposed by Bonner and Bandurski (16) and French and Beevers (45). One is that an increase in adenylate nucleotides would result in an enhanced rate of respiration. In this investigation, there seemed to be a correlation between the tissue content of adenylate nucleotides and respiration. Tristeza inoculation of 'Key' lime tissue increased both adenylate nucleotides and respiration during the early stages of infection. When the content of adenylate nucleotides in the tissue decreased in the later stages of infection, respiration also decreased.

SUMMARY AND CONCLUSIONS

To obtain a better understanding of a virus-host relation, a study was undertaken of the influence of tristeza (severe strain, T_3), alone and in combination with gibberellic acid or thiouracil or both chemicals, on certain aspects of the physiology of Citrus aurantifolia Cv. 'Key' lime. The influence of these agents on growth and development, oxygen uptake, soluble and hydrolyzable carbohydrates, total nitrogen, phosphorus, potassium, calcium, free amino acids, and acid-soluble nucleotides of the host during the infection stage were determined.

Measurements of stem elongation indicated that a definite suppression of growth could be detected on the fourteenth day after infection. Gibberellic acid overcame this suppression of growth caused by the virus. The thiouracil, alone and in combination with the virus, had little influence on elongation. However, both gibberellic acid and thiouracil hastened the onset of visible symptoms commonly associated with tristeza infection.

During the early stages of infection, total oxygen uptake of the diseased tissues was much greater than that of non-diseased tissues. Maximum oxygen consumption was noted on the seventh day after inoculation. Thereafter, oxygen consumption of the infected tissue steadily declined, until on the thirty-fifth day it was significantly less than

that of virus-free material. Viral invasion of plant host was also accompanied in the first 21 days by an increase in total phosphorus, but in the later stages of infection it was significantly less in the virus-infected plants than in the control. In the case of total nitrogen, alcohol-soluble sugars, potassium, and calcium, there was little difference between the infected and non-infected tissues during the initial stages of infection. However, after 28 days of infection there was a significant decrease in the total nitrogen, carbohydrates, potassium, and calcium.

Oxygen uptake, alcohol-soluble carbohydrates, total nitrogen, phosphorus, calcium, and potassium were changed measurably when virus-infected citrus was treated with either gibberellic acid or thiouracil. An analysis of the changes noted indicated that substrate was not limiting in virus-infected tissue. It was suggested that other mechanisms, possibly an upset in the hormonal balance within the plant, could be responsible for hastening and intensifying the viral symptoms associated with tristeza.

Tests of the stability and transmissibility of the chemical-induced-intensified symptoms indicated that they were reproducible even after the third successive inoculation. Additional tests on the influence of different gibberellic acid concentrations on the reaction between tristeza and 'Key' lime indicated that gibberellic acid at concentrations of 10^{-3} M, and 10^{-4} M accelerated and intensified the onset of symptoms, but a 10^{-5} M concentration of GA actually suppressed the symptoms.

Changes in amino acids and acid-soluble nucleotides as the result of tristeza infection were determined from the time of infection until visible symptoms of the virus were evident on the plants. With the aid of paper chromatography, cysteine, glycine, serine, histidine, lysine, arginine, citrulline, tryptophan, and proline were found to be present in an 80 per cent ethyl alcohol extract of the 'Key' lime leaves. Comparisons, based on visual observations of area and intensity of the color of the spots, between chromatograms of extracts from infected and non-infected tissues showed that lysine was absent in the infected tissue on the seventh day after infection and reappeared again on the twenty-eighth day after infection, but still at an amount much less than in the control. Also, serine, glycine, and tryptophan were reduced considerably in the infected tissue on the seventh and twenty-eighth day after inoculation.

Using column chromatographic and spectroscopic techniques, it was found that soluble nucleotides increased in the early stages of viral invasion and decreased in the later stages. On the seventh day after infection ATP, ADP, AMP, UTP, UMP, GTP, GDP, CTP, and CMP were present in lesser quantities in infected than in non-infected leaves. However, on the fourteenth day after infection all acid-soluble nucleotides were significantly less in the virus-infected than in the non-infected leaves, except CMP. Then, in the later stages of infection, the quantity of any soluble nucleotide was less in the viral-infected tissue than in normal tissue.

BIBLIOGRAPHY

1. Allison, R. M. 1953. Effect of leaf-roll virus infection on soluble nitrogen composition of potato tubers. Nature 171: 573.

2. Andreae, W. A., and K. L. Thompson. 1950. Effect of leaf-roll virus on amino acid composition of potato tubers. Nature 166: 72-73.

3. Arnon, D. I. 1953. The physiology and biochemistry of phosphorus in green plants. In Soil and Fertilizer Phosphorus in Crop Nutrition. Eds. Pierre, W. H., and A. G. Norman. Acad. Press. New York. pp. 1-42.

4. Bacher, J. E., and F. W. Allen. 1950. The composition of certain fractions obtained by the action of ribonuclease on ribonucleic acid from yeast. J. Biol. Chem. 183: 633-639.

5. Baddiley, J. 1955. Nucleosides and Nucleotides. In Nucleic Acids. Eds. Chargaft, E., and J. N. Davidson. Acad. Press. New York. pp. 137-188.

6. Bailova-Yankulova. 1961. Free amino acids in potato tubers of plants attacked by a virus. Compt. Rend. Acad. Bulgare Sci. 14: 515-518.

7. Barton, L. V. 1956. Growth response of physiological dwarfs of Malus arnoldiana Sarg. to gibberellic acid. Contrib. Boyce Thompson Inst. 18: 311-317.

8. Basler, E., and B. Commoner. 1956. The effect of tobacco mosaic virus biosynthesis on the nucleic acid content of tobacco leaf. Virology 2: 13-28.

9. Bawden, F. C., and B. Kassanis. 1954. Some effects of thiouracil on virus-infected plants. J. Gen. Microbiol. 10: 160-173.

10. _____ and A. Kleczkowski. 1957. An electrophoretic study of sap from uninfected and virus-infected tobacco plants. Virology 4: 26-40.

11. _____ and N. W. Pirie. 1952. Physiology of virus diseases. Ann. Rev. Plant Physiol. 3: 171-188.

12. Belkengren, R. O. 1961. Western-X-little cherry virus effects on respiration of host tree. Plant Physiol. 36: 827-828.

13. Bell, A. A. 1962. Respiratory changes associated with rust and virus diseases of Phaseolus vulgaris. Dissertation Abstr. 22: 2544.

14. Bennett, C. W., and A. S. Costa. 1949. Tristeza disease of citrus. J. Agr. Res. 78: 207-237.

15. Bhargawa, K. W. 1951. Some properties of four strains of cucumber mosaic virus. Ann. Appl. Biol. 38: 377-387.

16. Bonner, J., and R. S. Bandurski. 1952. Studies on the physiology, pharmacology and biochemistry of auxins. Ann. Rev. Plant Physiol. 3: 58-86.

17. Brewer, P. H., J. B. Kendrick and M. W. Gardner. 1926. Effect of mosaic on carbohydrate and nitrogen content of the tomato plant. Phytopath. 16: 843-851.

18. Brian, P. W., and H. G. Hemming. 1957. A relation between the effects of gibberellic acid and indole acetic acids on plant cell extention. Nature 179: 417.

19. Burton, C. L. 1962. Symptomatic, respiration and biochemical responses of cucumber to scab (Cladosporium cucumerinum Ell. and Arth.) infection. Dissertation Abstr. 22: 4154.

20. Busby, J. N. 1953. Tristeza in Florida. The Citrus Industry 34: 5-7

21. Campbell, E. G. 1925. Potato leaf-roll as affecting the carbohydrate, water, and nitrogen content of the host. Phytopath. 15: 427-430.

22. Carrera, C. 1933. Informe preliminar sobre una enfermedad nueva comprobada en los citrus de Bella Vista (Corrientes). Argentina Min. de Agr. Bol. Mens. 34: 275-280.

23. Cavell, A. J. 1955. The colorimetric determination of phosphorus in plant material. J. Sci. Food Agr. 6: 479-480.

24. Cherry, J. H., R. H. Hageman, J. N. Rutger, and J. B. Jones. 1961. Acid soluble nucleotides and ribonucleic acid of different corn inbreds and single-cross hybrids. Crop Science 1: 133-137.

25. [illegible], [illegible], F. I. Collins, and Donna Flesher. 1961. Effect of X radiation on corn seeds. Plant Physiol. 36: 566-572.

26. Chessin, M. 1957. Growth substances and stunting in virus-infected plants. Proc. 3rd Conf. Pot. Virus Diseases: 80-84.

27. Cohen, M., and L. C. Knorr. 1954. Present status of the tristeza in Florida. Proc. Fla. State Hort. Soc. 66: 20-22.

28. Commoner, B. 1959. Biochemical basis of tobacco mosaic virus (TMV) infectivity. Proc. Interntl. Congr. Biochem. 7: 17-30.

29. _____, and E. Basler. 1956. Variations in nucleic acids composition of tobacco mosaic virus. Virology 2: 477-495.

30. _____, and P. M. Dietz. 1952. Changes in non-protein nitrogen metabolism during tobacco mosaic virus biosynthesis. J. Gen. Physiol. 35: 847-856.

31. _____, and F. Mercer. 1951. Inhibition of biosynthesis of TMV by thiouracil. Nature 168: 113.

32. _____, and V. Nehari. 1953. The effects of TMV synthesis on the free amino acid and amide composition of the host. J. Gen. Physiol. 36: 791-805.

33. _____, D. L. Scheiber, and P. M. Dietz. 1953. Relationships between tobacco mosaic virus biosynthesis and the nitrogen metabolism of the host. J. Gen. Physiol. 36: 807-830.

34. _____, and J. Symington. 1962. Linear biosynthesis of tobacco mosaic virus incorporation of C^{14} into rods of different length. Proc. Natl. Acad. Sci. U.S. 48: 1984-1991.

35. Cooper, W. D., and H. S. Loring. 1954. The purine and pyrimidine composition of the TMV and the Holmes masked strains. J. Biol. Chem. 211: 505-515.

36. Crick, F. H. C. 1957. Nucleic acids. Scientific American September 1957.

37. Dahlstrom, R. V., and M. R. Shaft. 1961. Relationship of gibberellic acid to enzyme development. Adv. in Chem. Ser. No. 28., Am. Chem. Soc. pp. 49-58.

38. De Fremery, D., and C. A. Knight. 1955. A chemical composition of three strains of tomato bushy stunt virus. J. Biol. Chem. 214: 559-566.

39. Dickson, R. C., R. A. Flock, and D. Mc Melta. 1951. An insect vector of citrus quick decline disease. Calif. Citrog. 36: 135, 169-170.

40. Diener, T. O., and C. A. Dekker. 1954. Isolation and identification of l-pipecolic acid from western-X-diseased peach leaves. Phytopath. 44: 643-645.

41. Duncan, D. B. 1955. Multiple range and multiple F test. Biometrics 2: 1-42.

42. Dunlap, P. A. 1930. The total nitrogen and carbohydrates and relative rates of respiration in virus-infected plants. Am. J. Bot. 17: 348-357.

43. _____. 1931. Carbohydrate variations accompanying the mosaic disease of tobacco. Am. J. Bot. 18: 328-336.

44. Fawcett, H. W., and J. M. Wallace. 1946. Evidence of virus nature of citrus quick decline. Calif. Citrog. 32: 50, 88-89.

45. French, R. C., and H. Beevers. 1953. Respiration and growth responses induced by growth regulators and allied compounds. Am. J. Bot. 40: 660-666.

46. Fraenkel-Conrat, H. 1956. The role of the nucleic acid in the reconstitution of active tobacco mosaic virus. J. Am. Chem. Soc. 78: 882-883.

47. _____, and B. Singer. 1957. Virus reconstitutions: II. Combination of protein and nucleic acid from different strains. Biochim. et Biophys. acta. 24: 540-548.

48. _____, _____, and R. C. Williams. 1957. Infectivity of viral nucleic acid. Biochim. et Biophys. acta. 25: 87.

49. Francki, R. I. B. 1962. Inhibition of plant virus multiplication in two host species by 2-thiouracil. Virology 17: 1-8.

50. _____. 1962. Relation between incorporation of 2-thiouracil into tobacco mosaic virus nucleic acid and virus inhibition. Virology 17: 22-29.

51. _____, and R. E. F. Mathews. 1961. Stimulation of turnip-yellow mosaic virus protein produced by 2-thiouracil. Nature 191: 1078-1080.

52. Filippo, M. G., and M. Grilli. 1960. Free amino acids in potato (Solanum tuberosum) tubers from healthy and virotic clones. Pubbl. Univ. Cattolica S. Cuore, Ann., fac. Agrar. 78 No. 2: 13-28. Chem. Abstr. 55: 10591 I.

53. Galston, A. W., and W. K. Purres. 1960. The mechanism of action of auxin. Ann. Rev. Plant Physiol. 11: 239-276.

54. Gierer, A., and K. W. Mundry. 1958. Production of mutants of tobacco mosaic virus by chemical alteration of its ribonucleic acid in vitro. Nature 182: 1457-1458.

55. _____, and G. Schramm. 1956. Infectivity of ribonucleic acid from TMV. Nature 177: 702.

56. Gordon, M. P. 1959. The utilization of exogenous adenine and uracil from the synthesis of tobacco mosaic virus nucleic acid. Virology 9: 492-494.

57. Govindjee, M. M. Laloraya, and T. R. Rao. 1956. Formation of asparagine and increase in the free amino acid content of virus-infected leaves of Abelmoschus esculentus. Experientia 12: 180.

58. Grant, T. J. 1952. Evidence of tristeza, or quick decline, virus in Florida. Proc. Fla. State Hort. Soc. 65: 28-31.

59. _____. 1957. Tristeza virus strains. Citrus virus diseases. Proc. Conf. Citrus Virus Diseases 1957: 45-55.

60. _____, and A. S. Costa. 1948. A progress report on studies of tristeza disease of citrus in Brazil. I. Behavior of a number of citrus varieties as stocks for sweet orange and grapefruit, and as scions over sour orange rootstock when inoculated with tristeza virus. Proc. Fla. State Hort. Soc. 61: 20-33.

61. _____, and _____. 1951. A mild strain of tristeza virus of citrus. Phytopath. 41: 114-122.

62. _____, and P. Higgins. 1957. Occurrence of mixtures of tristeza virus strains in citrus. Phytopath. 47: 272-276.

63. _____, and W. Jones, and G. G. Norman. 1959. Present status of heat treatment of citrus viruses. Proc. Fla. State Hort. Soc. 72: 45-48.

64. Grieve, B. J. 1943. Studies in the physiology of host-parasite relations. 4. Some effects of tomato spotted wilt on growth. Australian J. Exptl. Biol. Med. Sci. 21: 89-101.

65. Grigsby, B. H. 1938. Physiological investigations of red raspberry plants inoculated with red raspberry mosaic. Mich. State Bull. 160.

66. Hart, R. G. 1957. On the distribution of purine and pyrimidine bases in the nucleic acid of TMV. Proc. Natl. Acad. Sci. U.S. 43: 457-463.

67. Henderson, F. J., and G. A. Le Page. 1958. Naturally occurring acid-soluble nucleotides. Chem. Rev. 58: 645-688.

68. Holden, M., and M. V. Tracey. 1948. The effect of infection with tobacco mosaic virus on the leaves of nitrogen, phosphorus, protease, and pectase in tobacco leaves and on their response to fertilizers. Biochem. J. 43: 151-156.

69. Hughes, W. A., and C. A. Lister. 1949. Lime disease in the Gold Coast. Nature 164: 880.

70. Jacobs, W. P. 1959. What substances normally control a given biological process? 1. Formation of some rules. Dev. Biol. 1: 527-533.

71. Jodidi, S. L., S. C. Moulton and K. S. Markley. 1920. The mosaic disease of spinach as characterized by its nitrogenous constituents. J. Am. Chem. Soc. 42: 1883-1892.

72. Johnson, M. J. 1941. Isolation and properties of a pure yeast polypeptidase. J. Biol. Chem. 137: 575-586.

73. Kleber, W., M. Lindemann and P. Schmid. 1959. Micro-malting tests with gibberellic acid. Braunwelt 99: 1781.

74. Knight, C. A. 1952. The nucleic acids of some strains of TMV. J. Biol. Chem. 197: 241-249.

75. Knorr, L. C. 1956. Suscepts, indicators, and filters of tristeza virus, and some differences between tristeza in Argentina and in Florida. Phytopath. 46: 557-560.

76. Kurtzman, R. H., A. C. Hildebrandt, R. H. Burris, and A. J. Riker. 1960. Inhibition and stimulation of TMV by Purines. Virology 10: 432-448.

77. Kutsky, R., and T. W. Rawlins. 1950. Inhibition of virus multiplication by naphthalene acetic acid in tobacco tissue. J. Bact. 60: 763-766.

78. Lalorya, M. M., and Govindjee. 1955. Effect of "tobacco leaf-curl" and tobacco mosaic virus on the amino acid and amide content of nicotiana sp. Nature 175: 907-908.

79. Limasset, P., F. Levieil, and M. Sechet. 1948. Influence of phytohormone on the development of viruses X and Y in tobacco. Compt. Rend. Acad. Sci. 227: 643-645.

80. Loebenstein, G. 1959. Effect of a virus infection in sweet potatoes upon the host plant respiration. Virology 9: 62-71.

81. Loring, H. S. 1942. Reversal inactivation of tobacco mosaic virus by crystalline ribonuclease. J. Gen. Physiol. 25: 497-505.

82. Magdoff, B. M., Richard J. Block, and D. B. Montie. 1956. Amino acid composition of southern bean mosaic virus. Contrib. Boyce Thompson Inst. 18: 371-376.

83. Maramorosch, K. 1957. Reversal of virus-caused stunting in plants by gibberellic acid. Science 126: 651.

84. Marcel, B., and G. Catayee. 1961. Carbohydrate metabolism of branches of healthy grapevines and those infected with "court-noues" virus disease. Compt. Rend. Soc. Biol. 155: 327-8. Chem. Abstr. 56: 726 f.

85. Markham, R., and J. D. Smith. 1950. Chromatographic studies on nucleic acids. 3. The nucleic acid of five strains of TMV. Biochem. J. 46: 513-517.

86. Martin, L. F., A. K. Balls, and H. H. McKinney. 1938. The protein content of mosaic tobacco. Science 87: 329.

87. Meneghini, M. 1946. Sobre a natureza e transmissibilidade da doença "tristeza" dos citrus. O Biologico 12: 285-287.

88. _____, and C. C. Delwiche. 1951. The multiplication of TMV in the host tobacco plant. J. Biol. Chem. 189: 177-186.

89. Mercer, F. L., T. E. Lindhorst, and B. Commoner. 1953. Inhibition of tobacco mosaic virus biosynthesis by 2-thiopyrimidine. Science 117: 558-559.

90. Miller, G. L., and W. M. Stanley. 1942. Derivatives of tobacco mosaic virus. II. Carbobenzoxy, p-chlorobenzoyl and benzene sulfonyl virus. J. Biol. Chem. 146: 331-338.

91. Millerd, A., and K. J. Scott. 1962. Respiration of the diseased plant. Ann. Rev. Plant Physiol. 13: 559-574.

92. Moreira, S. 1942. Observanções sobre a "tristeza" dos citrus, ou "podridão das radicelas". Biológica 8: 269-272.

93. Morgan, D. G., and G. C. Mees. 1956. Gibberellic acid and the growth of crop plants. Nature 178: 1356-1357.

94. Motirmani, D. P. 1961. Effect of variation in concentration of K and Ca in nutrient media on the growth, nucleotides, nucleic acid, and protein content of young corn plants. Dissertation Abstr. 21: 2846.

95. Nelson, N. 1944. A photometric adaptation of the somogyi method for the determination of glucose. J. Biol. Chem. 153: 375-380.

96. Norman, G. G. 1959. Florida State Plant Board Program for virus-free budwood. Proc. Conf. Citrus Virus Diseases 1957: 237-242.

97. Oberholzer, P. C. J. 1947. The bitter-seville rootstock problem. Farming in S. Africa 22: 489-495.

98. O'Reilly, H. J. 1953. Physiological changes induced in peach foliage by western-X-disease virus. (Abs.) Phytopath. 43: 481.

99. Orlob, G. B., and D. C. Arny. 1961. Some metabolic changes accompanying infection by barley yellow dwarfs. Phytopath. 51: 768-775.

100. Owen, P. C. 1955. The respiration of tobacco leaves in the 20-hour period following inoculation with TMV. Ann. Appl. Biol. 43: 114-121.

101. _____. 1955. The respiration of tobacco leaves after systemic infection with tobacco mosaic virus. Ann. Appl. Biol. 43: 265-272.

102. _____. 1956. The effect of infection with tobacco mosaic virus on the respiration of tobacco leaves of varying ages in the period between inoculation and systemic infection. Ann. Appl. Biol. 44: 227-232.

103. _____. 1957. The effect of infection with tobacco Etch virus on the rates of respiration and photosynthesis of tobacco leaves. Ann. Appl. Biol. 45: 327-331.

104. Paleg, L. G. 1960. Physiological effects of gibberellic acid. II. on starch hydrolyzing enzymes of barley endosperm. Plant Physiol. 35: 902-906.

105. _____. 1961. Physiological effects of gibberellic acid. III. observations on its mode of action on barley endosperm. Plant Physiol. 36: 829-837.

106. Pavillard, J. 1954. La teneur en auxine des pommes de terre virosées. Proc. 2nd. Conf. Pot. Virus Diseases: 178-184.

107. Perdrizet, E. 1961 a. Comparative study of the metabolism of sugar in healthy potatoes and those attacked with the leaf-roll virus. Compt. Rend. 1961. 252: 2288-2290.

108. _____. 1961 b. Comparison of the metabolism of free amino acids in the potato that a healthy and those attacked with the roll virus. Compt. Rend. 1961. 252: 2756-2758.

109. Peterson, P. D. 1931. Plastid pigments and chlorophyllase contents of tobacco as influenced by three types of mosaic. Phytopath. 21: 119.

110. Porter, C. A. 1959. Biochemistry of plant virus infection. Advances in Virus Res. 6: 75-91.

111. _____, and L. H. Weinstein. 1957. Biochemical changes induced by thiouracil in cucumber mosaic virus-infected and non-infected tobacco plants. Contrib. from Boyce Thompson Inst. 19: 87-106.

112. _____, and _____. 1961. Incorporation of 2-thiouracil-s^{35} into RNA and acid soluble nucleotides of vamorr 48 tobacco. Virology 15: 505-507.

113. Reddi, K. K., and C. A. Knight. 1956. Structural studies on the nucleic acid of some strains of tobacco mosaic virus. I- The residual material after RNase digestion. J. Biol. Chem. 221: 629-633.

114. Rohrbaugh, P. W., L. J. Klotz, and G. A. Zentmyer. 1951. A Progress Report on Quick Decline Studies. Part IV. The Effect of Quick "Decline" on the Respiration Rate. Calif. Citrog. 31: 199.

115. Rosa, J. T. 1927. Chemical changes accompanying the western-yellow-blight of tomato. Plant Physiol. 2: 163-169.

116. Sachs, R. M. 1961. Gibberellic, auxin, and growth retardant effect upon cell division and shoot histogenesis. Adv. in Chem. Ser. No. 28. Am. Chem. Soc. 59-70.

117. Schneider, H. 1954. Anatomy of bark of bud union, trunk, and roots of quick-decline-affected sweet orange trees on sour orange rootstock. Hilgardia 22: 567-581.

118. Selman, I. W., M. R. Brierley, G. F. Pegg, and T. A. Hill. 1961. Changes in the free amino acids and amides in tomato plants inoculated with tomato spotted wilt virus. Ann. Appl. Biol. 49: 601-615.

119. Skoog, F. 1954. Substances involved in normal growth and differentiation of plants. Brookhaven Symposia in Biology 6 (BNL 258): 1-21.

120. Sleeth, B. 1959. The citrus budwood certification program in Texas. Proc. Conf. Citrus Virus Diseases 1957: 232-326.

121. Steel, R. G. D., and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Company, Inc. New York. pp. 232-251.

122. Stewart, I. 1962. The effect of minor element on free amino acids in citrus leaves. Proc. Am. Soc. Hort. Sci. 81: 244-249.

123. Stowe, B. B., and T. Yamaki. 1957. The history and physiological action of the gibberellins. Ann. Rev. Plant Physiol. 8: 181-216.

124. Streets, R. B. 1959. Citrus bud certification in Arizona. Proc. Conf. Citrus Virus Diseases 1957: 243.

125. Takahashi, W. N. 1947. Respiration of virus-infected plant tissue and effect of light on virus multiplication. Am. J. Bot. 34: 496-500.

126. _____, and M. Ishii, 1952 a. An abnormal protein associated with tobacco mosaic virus infection. Nature 169: 419.

127. True, R. H., O. F. Black, and J. W. Kelly. 1918. Physiological studies of normal and blighted spinach. J. Agr. Res. 15: 369-408.

128. _____, and L. A. Hawians. 1918. Carbohydrate reduction in healthy and in blighted spinach. J. Agr. Res. 15: 381-384.

129. Umbriet, W. W. 1957. Manometric techniques. Umbriet, W. W., R. H. Burris, and J. F. Stauffer, Eds. Burgess Publishing Company. Minneapolis, Minn.: 1-17.

130. Vayonis, G. C. 1954. Phosphorus disturbances in mosaic virus infected tobacco plants. Physiol. Plantarum 7: 687-697.

131. Wallace, J. M. 1959. Tristeza disease investigations, an example of progress through cooperative international research. Proc. Conf. Citrus Virus Diseases 1957: 29-33.

132. Webber, H. J. 1925. A comparative study of the citrus industry of South Africa. Union S. Africa Dept. Agr. Bull. 6: 1-106.

133. _____. 1943. The "tristeza" disease of sour-orange rootstock. Proc. Am. Soc. Hort. Sci. 43: 160-168.

134. West, S. H. 1962. Protein, nucleotide, and ribonucleic acid metabolism in corn during germination under water stress. Plant Physiol. 37: 565-571.

135. Whitehead, T. 1931. Respiration of healthy and leaf-roll potatoes. Nature 128: 967.

136. Wildman, S. G., C. C. Cheo, and J. Bonner. 1949. The proteins of green leaves. III. Evidence of the formation of tobacco mosaic virus protein at the expense of a main protein component in tobacco leaf cytoplasm. J. Biol. Chem. 180: 985-1001.

137. Wittwer, S. H., M. J. Bukovac, and B. H. Grigsby. 1957. Studies in gibberellic acid. Mich. State Univ. Agr. Expt. Sta. Quart. Bull. 40: 1-203.

138. Woods, A. F. 1900. Inhibiting action of oxidase upon distase. Science 11: 17-19.

139. Wynd, F. L. 1943 a. Metabolic phenomenon associated with virus infection in plants. Bot. Rev. 9: 395-465.

140. _____. 1943 b. Respiration of mosaic-infected plants. Plant Physiol. 18: 90-98.

141. Yamaguchi, A. 1960. Increased respiration of leaves bearing necrotic local virus lesions. Virology 10: 287-293.

BIOGRAPHICAL SKETCH

The author, Kunwar Brijendra Pratap Singh, was born on October 10, 1938, in the district of Fatehpur, India. He passed his high school examination of Uttar Pradesh Board of High School in June, 1953. In 1953, he was transferred to Government Agriculture College Kanpur (affiliated with Agra University), from which he received the degrees of Bachelor of Science in Agriculture, in 1958, and Master of Science in Agriculture, with horticulture as a major subject, in 1960.

He entered the University of Florida, Gainesville, in September, 1960. During the period of his studies, he was teaching and research assistant in the Department of Fruit Crops. He completed his work toward the degree of Doctor of Philosophy in the Department of Fruit Crops in August, 1963.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of the committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council and was prepared as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August, 1963

Dean, College of Agriculture

Dean, Graduate School

SUPERVISORY COMMITTEE

Chairman

Co-Chairman